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REVIEW ARTICLE

BILE SALTS—THEIR PHYSIOLOGICAL FUNCTIONS IN THE GASTROINTESTINAL TRACT

Bile salts and related compounds (bile alcohols) are present in the digestive tract of all vertebrates. The comparative chemistry of these compounds has been elucidated by Haslewood, the C_{24} acids being the latest step in the evolution (25). The bile salts belong to the class of detergents, that is to say their molecules possess both fat and water-attracting regions, which give them special properties in water solution. Other types of detergents have been found in the gastric juice of some crustaceans (crab lobster crayfish); these are composed of medium chain fatty acids acylated to one or two amino acids, one example being the acyl-sarcosyl-taurine of the crab (37,54).

The universal occurrence of detergents in the gastrointestinal tract points to their great physiological importance in spite of which, up to the last decade, the function of the bile salts in the intestinal tract has been rather vaguely formulated as an aid in the emulsification of the dietary fat and as activators for lipase. It seems now clear however that, besides having general detergent properties, the bile salts also have certain rather specific functions in the intestinal tract which will be discussed here.

CHEMISTRY—PHYSICAL CHEMISTRY—METABOLISM

Chemistry The bile salts are formed in the liver from cholesterol and contain the well recognized steroid skeleton (3 5). The conversion of cholesterol to bile salts involves a series of chemical transformations the mechanism of which has been elucidated in great detail (4). The two bile acids primarily formed in man are the 3 7 12-trihydroxycholanic acid called cholic acid and the 3,7-dihydroxycholanic acid called chenodeoxycholic acid. Two secondary bile acids, 3 12-dihydroxy- and 3-monohydroxycholanic acid with the trivial names deoxycholic and lithocholic acid, are formed from the primary bile acids in the intestinal tract by the action of bacteria which remove the OH group in the 7α-position, of these only deoxycholic acid is reabsorbed and excreted to the bile in measurable quantities. In the bile the bile acids are present in conjugated form with glycine or taurine to form cholyl taurine, deoxycholylglycine etc.

Physical chemistry In water solution bile salts (in the following bile salts refer to salts of the conjugated bile acids) have detergent properties (30, 36). At low concentrations they form molecular dispersed solutions. At a certain concentration that is characteristic for each bile salt the bile salt molecules, due to their content of hydrophilic and hydrophobic centers, start to form aggregates called micelles. This concentration is usually referred to as the critical micellar concentration (CMC) but in general is more of a critical micellar range. Detergents added to the solution in excess of this range all form aggregates and the molecular dispersed phase which therefore has a constant concentration, enters into rapid equilibrium with the micelles which form the micellar phase (Fig. 1). The size and shape of the micelles vary with the type of bile salt, the trihydroxy conjugates under physiological conditions forming aggregates with 4–8 molecules, the dihydroxy bile salts having aggregation numbers of the order of 16–25. From a physico-chemical point of view the bile salts behave in principle similarly to other anionic detergents. Bile salt solution has a much lower solubilizing capacity for non-polar lipids than ordinary paraffin chain detergents; the capacity of bile salt solutions to solubilize insoluble amphiphilic lipids such as monoglycerides into mixed micelles is, on the other hand, much greater. The ability of these mixed bile salt micelles to solubilize non-polar lipids is comparable to that of the ordinary aliphatic chain detergents.

Metabolism Bile acids are synthesized in the liver from cholesterol, conjugated with taurine or glycine and excreted in the bile (7). A most important feature of bile salts is that they undergo an enterohepatic circulation with a rather efficient reabsorption normally better than 98 % (68). The total pool of bile salt in man is of the order of 2.5–4.5 g (42, 66). When the gastrointestinal tract is at rest, this pool is almost entirely concentrated in the gallbladder and the bile secreted from the liver is low in bile salt. After food intake the gallbladder contraction forces the bile salt pool into circulation and the bile salts are reabsorbed and re-excreted in the liver bile as long as digestion takes

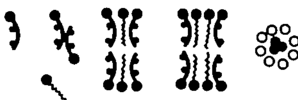


Fig 1 Structures present in a solution of bile salts above the critical micellar concentration in the presence of another amphiphilic molecule. The molecular dispersed phases are considered to be in equilibrium with the micellar phase.

place. The extent of recirculation of the bile salt pool depends on the number of meals and their size and composition and it is considered that the pool circulates 6–10 times a day (8). The absorption of bile salts is passive in the jejunum and upper ileum (mostly glycine conjugates) and active in the lower ileum (glycine and taurine conjugates). For every circulation some bile salt is normally lost to the colon. Absorption here is slight and bacterial degradation of the bile salt molecule takes place resulting in large number of different metabolites, most of which are excreted by the feces. Deconjugation and 7α-dehydroxylation are the most important processes resulting in the formation of free lithocholic acid (3-OH cholanic acid) from chenodeoxycholic acid and free deoxycholic acid (3 12-OH-cholanic acid) (from cholic acid).

The excretion of bile salts via the feces is around –800 mg/day giving a half life of the bile salts in the order of 3 days. Each individual bile salt molecule synthesized in the liver thus undergoes 15–20 enterohepatic circulations before it is lost via the feces (66). extent to which the bile salts are deconjugated i normal enterohepatic circulation has been estimated to be 10%/day for the taurine (28 63) and appreciably more for the glycine conjugates (27 53).

The different kinetic parameters of bile salt metabolism—pool size rate of hepatic synthesis and the enterohepatic cycling—can be measured by chemical methods and by the use of labeled bile acids. Variations in these parameters during different diseased states are of great diagnostic importance (34). The chief parameter of interest for digestion and absorption is the actual bile salt concentration in the content of the upper small intestine during digestion which can be sampled and determined. After a peak value during the first 20–30 min after food intake due to the emptying of the gallbladder the recirculated liver bile gives a concentration of bile salts in the intestinal content of 4–16 mM (8 61). This concentration is above the CMC of the mixed bile salt solution and a micellar phase is present in the intestinal content under normal conditions. The micellar phase of intestinal content can be obtained through ultracentrifugation or filtration through membrane filters (33 55). When the bile salt concentration is decreased below this level a micellar phase defect occurs which in general results in malabsorption (57).

The synthesis of bile salts by the liver is regulated by the amounts of bile salt returning to the liver. The maximum capacity of the human liver to synthesize bile salt is considered to be 4–6 times that of the normal level (21).

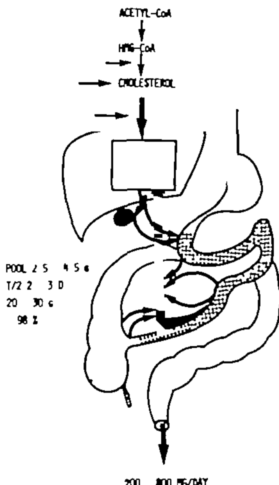


Fig 2 The metabolism of bile salts in essence. After being formed from cholesterol in the liver or reabsorbed from the blood by the liver the bile salt molecules are secreted to the bile discharged to the small intestine from which after having fulfilled their physiological function they are absorbed actively by the distal ileum to the portal circulation reexcreted by the liver etc. The amounts of bile salts excreted by the feces, which normally constitute a minor fraction of the totally circulated bile salts are compensated for by de novo synthesis in the liver from cholesterol. The de novo synthesis of bile salts in the liver is under negative feedback control by returning bile salt.

ROLE OF BILE SALTS FOR DIGESTION AND ABSORPTION OF FATS

The digestive enzyme

Pancreatic lipase. It has long been considered that bile salts are activators for pancreatic lipase. Recent investigations, however have shown that bile salts rather are inhibitors of this enzyme when it is present

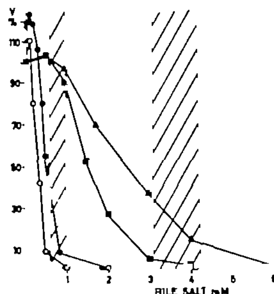


Fig. 3. Effect of bile salts on the lipase-catalyzed hydrolysis of tributyrine (*V* = rate of hydrolysis). Effect of two dihydroxyconjugates: chenodeoxycholyl taurine (○), deoxycholyl taurine (●), and two trihydroxyconjugates: cholyl taurine (■), cholyl glycine (▲), ▨ = the critical micellar range of the di- and tri-hydroxy bile salts, respectively.

in pure form (9, 10, 45). Inhibition of lipase by bile salts starts already at low bile salt concentrations and becomes complete when the critical micellar range is reached (Fig. 3). Other anionic detergents, such as dodecyl sulphate and acyl taurates, also inhibit lipase. This effect cannot therefore be considered specific for bile salt.

Bile salts belong to the so-called "mild detergents" group which only interact with proteins at specific sites, in contrast to the denaturing detergents of the dodecyl sulphate type, which also show so-called co-operative binding on the proteins, leading to conformational changes and denaturation with loss of biological activity (26, 44). Bile salts seem to stabilize the lipase and prevent it from being inactivated on the substrate surface (11).

It seems strange that bile salts, present in the intestinal content of all vertebrates in a concentration above the CMC, should inhibit the enzyme present from hydrolyzing the main dietary lipid component, the triglyceride. The lipolytic activity of pancreatic juice, however, is not inhibited by bile salt and it has been found that pancreatic juice contains a co-factor which is able to reactivate lipase in the presence of bile salts (9, 10, 45, 51) (Fig. 4). This co-factor, which has been named co-lipase (46), has been purified and is a polypeptide of molecular weight ≃ 11 000 with five disulphide bridges (16, 17, 46). The mechanism of action of co-lipase is not fully understood, but evidence indicates that the co-lipase–lipase interaction is entirely physico-chemical, i.e. hydrophobic and/or electrostatic, and that the complex formed has modified physico-chemical properties, which makes a proper interaction with the substrate possible.

The interaction with the lipase/co-lipase system is not specific for bile salts. It is of interest that the detergents shown to occur in the digestive juice of the Crustacea of the acyl amino acid or acyl dipeptide type also interact in a similar way with lipase/co-lipase (10).

Sterol ester hydrolase of pancreatic origin. This enzyme, which has been shown to be much less specific than the name implies, catalyzes the hydrolysis of a wide range of substrates and its activity is greatly stimulated by bile salts (1, 50). It has been shown that this enzyme forms an enzyme bile salt complex involving a reversible formation of a hexamer which may be the catalytically active form (38). This effect seems to be specific for bile salts and not given by other types of detergents.

Pancreatic phospholipase A_2. This enzyme is present in the pancreatic juice as a zymogen or proenzyme

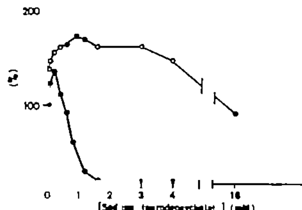


Fig. 4. Lipase activity (*V*) is inhibited by increase in concentration of deoxycholyl taurine over the critical micellar concentration (●). Co-lipase prevents the inhibition of lipase (○). The experiment was performed at pH 7.0 in 150 mM NaCl (10).

LIPID CLASSES

NON POLAR

POLAR

INSOLUBLE NON SWELLING AMPHIPHILES

INSOLUBLE SWELLING AMPHIPHILES

SOLUBLE AMPHIPHILES

STEROL ESTERS → STEROLS → FATTY ACIDS - - - - → SOAP
VITAMIN ESTERS → VITAMINS → FATTY ACIDS - - - - → SOAP
TRIGLYCERIDE → FATTY ACIDS - - - - → SOAP
TRIGLYCERIDE → MONOGLYCERIDE

PHOSPHOLIPID → LYSOPHOSPHOLIPIDS
PHOSPHOLIPID → FATTY ACID - - - - → SOAP

BILE SALTS

Fig 5 A physico-chemical lassification of dietary lipids and the hydrolytic product formed by the action of pancreatic enzymes thereupon The broken lines be tween fatty acid and soap indicate that this conversion most probably is unimportant at the pH alues of intestinal content The lipids belonging t the class of soluble amphiphiles form mi ed micelles with the bile salts. F tty acid soaps have higher micellar solubility than the unionized fatty acids even though it is considered that micellar solubilization into bile salt micelles depresses the ionization of the fatty acid

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The phosphoglycerides with long chain fatty acids, as they occur as components of dietary lipid show an absolute equirement for bile salt with thi enzyme phoglycerides with shorter chain fatty acids such lecithin w th C_{8}–C fatty acid esters, however hydrolyzed by phospholipase without addition of bile salt (43) The importance of bile salt for the hydrol si of the long chai phospholipids may be seen a solubilization of the substrate nto mixed bile salt-phosphoglyceride micelles The bile salts thus do not act a co-factor for the enzyme in the normal sense

Bile salt have been tested as activators of other digestive enzymes but with no positi results (43) with the possible xception of enterokinase (.3 52).

The role f bile salts in the solubilization of the products of the luminal digestion

Dietary lipids make p a very heterogeneous group of compounds from a chemical point of view In a discussion of their behavior in the intestinal lumen they can more conveniently be classified according to their interaction with water and bile salt solutions (12, 36). Lipids can then be defined as molecules containing a hydrophobic hydrocarbon keleton, which to different extents is substituted by polar (hydrophilic) groups. It is the balance between these two parts which determines the behavior of a l pid in water and bile salt solution. Fig. 5 gives a summary of the classification of important dietary lipids based on their behavior in water solution. It can be seen that the main components of the dietary fat, triglycerides with long chain fatty acid, are classified as water-insoluble non-swelling amphiphiles. They disperse in bile salt solutions forming emulsions, but their micellar solubility in bile salt solution i very low Most of the minor components of the dietary lipid such as sterols and sterol esters, fat-soluble vitamins, hydrocarbon etc., show interactions with water and bile salt solutions similar to those of the triglyceride

Other components of the dietary fat of interest in this spect are the phospholipids. Although this group of lipids for practical purposes is also insoluble in water its members contain enough polar groups to interact with water Water will penetrate into part of the molecules and cause them t swell and they have been defined a insoluble welling amphiphiles. These lipids interact with bile salt solutions to form mixed micellar solutions as previously discussed. The effect of bile salts on the dietary lipids is thus an effect of their general detergent properties, forming an emulsion in equilibrium with mixed micellar solution, the partition of the different species between these two physical states being dependent on their non-polar/polar balance. Although most of the lipids of the dietary fat are classified water-insoluble ll these compounds are considered to have a definite but very low water solubility This third molecular dispersed phase which has been ascribed an important role in the absorption process, considered to be in equilibrium with the other phases to form a 3-phase emulsion-micellar-water system. The solubility of the long chain fatty acids in water is still low but several orders of magnitude higher than that of the corresponding alkane (7). The ioniza-

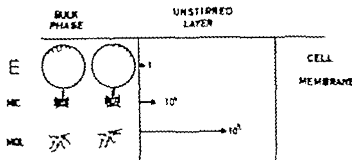


Fig. 6. Importance of the particle weight of lipids for their diffusion over the unstirred water layer covering the enterocyte cell membrane. *E*=emulsion droplet (chiefly triglyceride): diameter = μ, particle weight =4×10⁻¹² g, aggregation number =...4×10⁹. *MC*=micelle: diameter =40 Å, particle weight (non-hydrated) ≈4 10⁻¹⁹ g, aggregation number =100. *MOL*=molecular dispersed: molecular weight =250, weight of the single molecule =4 10⁻²² g, aggregation number =1. Rate of diffusion related to 1 M. The thickness of the unstirred layer is considered to be within the range of 5–70 10⁻ Å (6).

tion of the fatty acids also increases their water solubility (... 31).

The effect of the lipolytic enzymes in the intestinal content is to catalyze the formation of products which are more polar than the substrate and therefore have a higher degree of interaction with water and bile salt solutions (Fig. 5). This results in transfer of the product of lipolysis from the oil phase → the micellar phase → the aqueous phase. The products formed, with the exception of the lysophospholipids which have high water solubility, still have very low aqueous molecular solubility. The effect of bile salts in these systems is then to solubilize the polar products formed by lipolysis, mainly monoglyceride and fatty acid, into mixed micellar solutions. The effect of the micellar solubilization on the concentration of such compounds in the water phase has not been investigated due to lack of suitable methods.

The bile salts also affect the ionization of the fatty acids dissolved into the micelles. The co-operative effects of the solubilization of the hydrocarbon part of the fatty acid in the micellar interior and the presence of neighbouring charged bile salt molecules depress the ionization of the fatty acids, their apparent pKa are increased. This is most probably the mechanism by which bile salts in concentrations above their CMC affect the equilibrium composition of the product mixture catalyzed by pancreatic lipase so that more extensive hydrolysis (relatively more monoglyceride and fatty acid in the equilibrium mixture) is attained at the slightly acid pH values prevailing in the intestinal content (7).

Transport of lipids over the unstirred layer

It is one of the essential features of the micellar theory of fat absorption that micellar solubilization promotes fat absorption (32). Perfusion experiments in the rat (59) and in man (79) also indicate that this is the case. That triglyceride fat is hydrolyzed and absorbed to an appreciable extent in the absence of bile has been known for a long time and been explained by the hypothesis that, in the absence of bile, the low aqueous concentration of fatty acid is compensated by absorption over a longer period from a larger absorption area (39). The available experimental evidence has nonetheless led to the consideration that fatty acid and other lipid, even in the presence of bile salt, are absorbed from the molecular dispersed aqueous solution (60). The explanation of this apparent discrepancy may be found in the importance of the so-called unstirred layer in lipid absorption. The absorptive surface of the intestinal mucosa cell is considered to be covered by a layer of unstirred water through which the molecules to be absorbed have to pass by diffusion (69). It is furthermore considered that the diffusion rate through this unstirred layer is related to the concentration of the lipid in the molecular dispersed phase and that transport of lipids in the absence of bile salt therefore is extremely slow. The bile salt is considered to increase the transport rate over the unstirred layer by presenting the lipid in high concentration in micellar solution to the unstirred layer (56).

The diffusion rate of a molecule or particle is inversely related to the square root of the molecular weight. If we consider the diffusion rate of an emulsion droplet with a diameter of μ, a mixed bile salt micelle with a hydrated diameter of 40 Å and molecular dispersed fatty acid, it can be calculated that their relative diffusion rate will be approximately 10⁴, 10⁵ and 10⁶ respectively (Fig. 6). These figures are only relative, but it can most probably be considered that emulsion particles diffuse so slowly that flux of particulate lipid into the unstirred layer is very unimportant in the absorption process. The diffusion rate in the unstirred layer of a mixed bile salt micelle is approximately one order of magnitude below that of a molecule, but on the other hand, as discussed previously, the concentration of lipids in the micellar phase is in many cases several orders of magnitude above that in the molecular dispersed phase. The micellar lipids should thus serve as a pressure head for the molecular dispersed phase (Fig. 7). Stated in another way, the function of the micelles is to overcome the resistance of the unstirred layer.

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NON POLAR — POLAR

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PHOSPHOLIPID → LYSOPHOSPHOLIPIDS

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BILE SALTS

Fig. 5. A physico-chemical classification of dietary lipids and the hydrolytic products formed by the action of pancreatic enzymes thereupon. The broken lines between fatty acid and soap indicate that the conversion most probably is unimportant at the pH values of intestinal content. The lipids belonging to the class of soluble amphiphiles form mixed micelles with the bile salts. Fatty acid soaps have a higher micellar solubility than the unionized fatty acids, even though it is considered that micellar solubilization into bile salt micelles depresses the ionization of the fatty acid.

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The phosphoglycerides with long chain fatty acids as they occur as components of dietary lipid, show absolute requirement for bile salt with this enzyme. ...osphoglycerides with shorter chain fatty acids such ... lecithin with C_6–C fatty acid esters, however, ...e hydrolyzed by phospholipase without addition of bile salt (43). The importance of bile salt for the hydr... of the long chain phospholipids may be seen as a solubilization of the substrate into mixed bile salt-phosphoglyceride micelles. The bile salts thus do not act ... co-factor for the enzyme in the normal sense.

Bile salts have been tested as activators of other digestive enzymes but with no positive results (43), with the possible exception of enterokinase (23, 52).

The role of bile salts ... the solubilization of the products of the luminal digestion

Dietary lipid make up a very heterogeneous group of compounds from a chemical point of view. In a discussion of their behavior in the intestinal lumen they can more conveniently be classified according to their interaction with water and bile salt solutions (1..., 36). Lipids can then be defined as molecules containing a hydrophobic hydrocarbon skeleton which to different extents is substituted by polar (hydrophilic) groups. It is the balance between these two parts which determines the behavior of a lipid in water and bile salt solution. Fig. 5 gives a summary of the classification of important dietary lipids based on their behavior in water solution. It can be seen that the main components of the dietary fat, triglycerides with long chain fatty acid, are classified as water-insoluble non-swelling amphiphiles. They disperse in bile salt solutions forming emulsions, but their micellar solubility in bile salt solution is very low. Most of the minor components of the dietary lipids such as sterols and sterol esters, fat-soluble vitamins, hydrocarbon etc. show interactions with water and bile salt solutions similar to those of the triglyceride.

Other components of the dietary fat of interest in this respect are the phospholipids. Although this group of lipids for practical purposes is also insoluble in water its members contain enough polar groups to interact with water. Water will penetrate into part of the molecules and cause them to swell and they have been defined as insoluble swelling amphiphiles. These lipids interact with bile salt solutions to form mixed micellar solutions as previously discussed. The effect of bile salts on the dietary lipids is thus an effect of their general detergent properties forming an emulsion in equilibrium with a mixed micellar solution, the partition of the different species between these two physical states being dependent on their non-polar/polar balance. Although most of the lipids of the dietary fat are classified as water-insoluble all these compounds are considered to have a definite but very low water solubility. This third molecular dispersed phase which has been ascribed an important role in the absorption process is considered to be in equilibrium with the other phases to form a 3-phase emulsion-micellar water system. The solubility of the long chain fatty acids in water is still low but several orders of magnitude higher than that of the corresponding alkane (?). The ioniza-

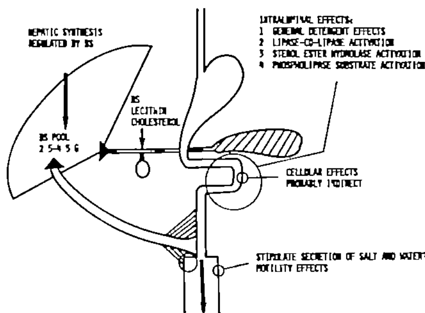


Fig. 8. Summary of the effects of the bile salts the gastrointestinal tract Bile salts also are of importance for the solubilization of lecithin and cholesterol of the bile, but thi function is not considered in this review

et al. (70) made the interesting observation that i the upper small intestine such secretion is abolished by the simultaneous presence of lecithi The inhibitory effect of lecithin may be due to its property of decreasing the actual concentration of bile salts in molecular form. The dihydroxy bile salts also cause water secretion from the intestine the secreted fluid is mainly sodium chloride solution and the effect is certainly not an osmotic effect. The physiological importance and the mechanism of this effect is unknown, but it has been suggested that physiological function of bile salts in the colon is to prevent undue dehydration of feces (19). When bile salt spills over into the large intestine due to incomplet absorption as seen after ileal resection and diseased states of the distal ileum, bile-acid-induced inhibition and secretion of water explains the diarrhea seen in these conditions (35). Bile salts also hav less well defined effects on gastric emptying (39), intestinal motility and the secretion of intestinal hormones (40)

TO WHAT EXTENT ARE THE FUNCTIONS OF BILE SALTS UNIQUE?

As noted, bile salts are detergent and the question is whether all the functions of the bile salts in the intestinal tract can be considered as general detergent effects or to what extent they can be said to be specific or unique for the bile salt. The bile salts can be said to have some special detergent properties. I general, they form smaller micelles than most other detergents and have a relatively high CMC, they show no cooperative binding to proteins (44) and therefor have no denaturing effects. They have a high capacity—in comparison with other detergents to dissolve water-insoluble, swelling amphiphiles such as monoglyceride and fatty acids into mixed micellar solutions—and low capacity to solubilize non-polar lipid The inclusion into the bile salt micelle of monoglyceride and long chain fatty acids, on the other hand, greatly increases the cosolubilization of non-polar lipids. These properties of the bile salt micelle are of particular importance for intestinal absorption, as they make lipolysi of paramount importance for the absorption of both the triglyceride fat and other lipids of dietary origin dissolved in the triglyceride. The products of lipolysis, the monoglycerides and fatty acids, may be said in thi way to provide solvent vehicle for other non-polar lipids, the absorption of which is dependent on the transport of fatty acids and monoglyceride in the micellar phase. Non-polar lipids such as hydrocarbons, fat-soluble vitamins, etc. when fed alone are scarcely absorbed at all from the intestinal tract. When fed together with triglyceride fat, appreciable quantities are absorbed due to their much greater solubility of this compound in the mixed bile salt micelles containing the hydrolytic products of the triglycerides. The extent of absorption of non-polar lipid therefore due to the special detergent properties of the bile salts, becomes dependent on its rate of absorption in relation to the triglyceride lipolytic product.

In vitro as well as in vivo experiments have de finitely shown that fatty acids can be absorbed when presented to the intestine in the form of micellar solution in non-bile salt detergent (13 67)

What, then, are the other functions of bile salt? As mentioned in the introduction, detergents other than those containing the steroid nucleus have been found in the digestive juice of some invertebrates, notably the Crustacea. This type of detergent, which is of the fatty acyl amino acid type, has been found to replace bile salts in the interaction with lipase/co-lipase, but thei ability to do so in other functions has not been investigated and no other detergent has so far been found to replace the bile salts in their function associated

with sterol ester hydrolase or phospholipase A_2. The bile salt therefore may be considered specific in these two functions, even though they are reflections of the detergent properties of the bile salt.

From a clinical point of view it would be of interest to have a substance that interfered with the functions of bile salt in the intestinal content. If a controlled malabsorption of fat could be established this could be of importance for the treatment of certain abnormalities of lipid metabolism. The only type of compound so far introduced for such purposes is insoluble cation exchangers such as cholestyramine and DEAE-Sephadex, which bind bile salts resulting in an increased fecal excretion and enhanced synthesis of bile acids from cholesterol (21). When given in adequate amount they also are able to produce a micellar phase deficit thereby leading to malabsorption. As bile salts are important for the function of the lipolytic enzymes in the intestinal tract another possibility would be to find substances which interfere with the function of these enzymes. *In vitro* the most potent inhibitors of pancreatic lipase are found among the cationic detergents exemplified by hexadecyl-(cetyl-)-trimethyl ammonium bromide. This compound inhibits lipase in concentration low enough to be practical to produce *in vivo*. Its general toxicity probably makes it useless for these purposes.

One remarkable feature of the metabolism of the bile salts in the vertebrates which is of paramount importance for their physiological function is their property to undergo an enterohepatic circulation. The importance of the enterohepatic circulation which is characterized by a distal active reabsorption of the bile salts is to maintain a high concentration of bile salt in the upper small intestine where absorption of the lipolytic products of the nutrients takes place, and to allow the daily circulation of bile salts in amounts greatly in excess of the pool size and the synthetic capacity of the liver. When the distal absorption of bile salt is not functioning, the bile salt pool may be washed out in a single passage of the intestinal tract, resulting in a micellar phase deficit. Some of the requirements for an active distal absorption related to the bile salt structure have been studied (41) but no substitute for the bile salt skeleton has been searched for.

CONCLUSION

The secretion of bile salts or analogues into the intestinal tract is a universal feature of all vertebrates, a fact that indicates a general importance of these substances for the function of the intestinal tract. Our present knowledge of the function of bile salts in the digestive tract can be summarized in the following points (Fig. 8).

1. A general detergent function: dispersing and solubilizing in the intestinal content dietary fats and their hydrolytic products in micellar form, thereby facilitating their uptake into the intestinal cell membrane.

2. Effects on the function of the lipolytic enzymes secreted in the pancreatic juice. (a) An interaction with pancreatic lipase and a polypeptide co-factor for this enzyme named co-lipase. (b) An interaction with sterol ester hydrolase to form a catalytically active enzyme/bile salt aggregate. (c) To disperse water-insoluble long chain phosphoglycerides in mixed micellar state so that they can serve as substrate for phospholipase A_2. (d) To activate enterokinase?

3. To stimulate the secretion of water and salt from the small and large intestine.

4. To affect the motility of the intestine and the secretion of intestinal hormones.

All recognized functions of bile salts for fat absorption take place in the intestinal content, outside the body. Effects of bile salt claimed to occur intracellularly in the enterocyte are most probably secondary to the intraluminal effects. The question has been considered as to what extent the functions of bile salts are general detergent effects or to what extent they can be considered unique.

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BLOOD PRESSURE AND BLOOD LIPIDS IN MEMBERS OF FAMILIES WITH A HEAVY AGGREGATION OF ESSENTIAL HYPERTENSION

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Abstract Eighty-four persons between 17 and 48 years of age who were siblings or children of hospitalized patients with essential hypertension and whose families had additional cases of high BP and/or manifestations, have been investigated. BP blood lipids and some other parameters were measured. As control group served 61 age-matched persons, randomly selected from a general health survey None of these controls had hypertensive disease in their families. Significantly higher BP was found in patients over the age of 33 with family history of hypertension than in the control group No difference below that age could be found. No signifi cant differences in blood lipid levels were found.

High blood pressure and its complications have long been observed to occur in certain families As early as in 1761 Morgagni described five cases of familial apoplexy (11) From then on there have been several reports of single families and studies of family histories (2 5 12 18) hypertensive patients being the pilot cases Most of these have indicated a familial factor in hypertension this factor has usually been considered to be mediated by a dominant gene (2 13 18) Others have been less definite about the role of heredity (19). Family histories taken from patients have often been suggested to stress the occurrence of the disease in relatives and may therefore present a false picture. In addition histories most often record arterial dis ease the cause of which is not always hypertension.

The criticisms mentioned were first put forward in 1954 by Hamilton et al. (8) who reported a study of BP measurements in a population sample in relatives of hypertensives and in relatives of controls. BP was shown to increase with age in all three samples though in the second group the recordings were skewed towards higher pressures than in the other groups in which they were rougly on the same level The authors concluded that BP is multifactorially determined and at least partly inherited. The nature of these factors has been discussed by several authors in terms of heredity and environment (4 7 10 14 19) most papers favouring heredity as being the principal contribution (4 10 14). Strong support has re cently been lent to this theory by investigations in early childhood which revealed clustering of raised BP within families (20)

The present study is an investigation of the BP level the blood lipid levels and certain other para meters of members of families apparently susceptible to essential hypertension and its complications We have found no earlier study in which families at high risk in this respect have been subjected to an investigation of this kind The aim from a practical point of view was to try to establish the age above which it would appear mandatory to supervise these individuals closely in order to detect the possible appearance of hyper tension.

MATERIAL

The names were collected of all patients admitted to the Medical Department of the University Hospital in Uppsala over the last three years who were registered as suffering from arterial hypertension shown to be of essential type in the cumulative diagnosis index. Their medical histories were studied for evidence of familial hypertension. Patients, whose case histories revealed one or more family members who had either raised BP detected before the age of 65 or had a stroke or developed cardiac insufficiency not due to organic valvular disease before their 70th birthday were questioned concerning siblings and children. Thus myocardial infarctions or hypertension in connection with



Fig 1 Age versus RSDP in males ■=siblings or children in families with at least two known members with hypertensive disease <65–70 years. □=controls from general health survey who denied a family history of hypertension and/or cardiovascular disease before the age of 65

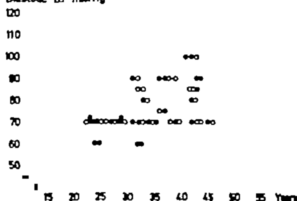


Fig 2 Age versus RSDP in females. ●=siblings or children in families with at least two known members with hypertensive disease <65–70 years O=controls from general health survey who denied family history of hypertension and/or cardiovascular disease before the age of 65

renal insufficiency in the history were not accepted as vidence of familial hypertensive disease and those patients were excluded from the tudy

All the relatives (84 person 39 men and 45 women aged between 17 and 49) thus collected were invited to participate in the investigation and to undergo an out patient examination. A thorough medical history was obtained from each No case of renal insufficiency was detected by this screening.

A control group of 61 persons, 77 men and 34 women were drawn consecutively from a group attend- a general health survey Each control subject was d with regard to age with one of the patient (±3 years at th time of examination). The con- group came from the same community as the pa- t group and the known presence of family members with hypertension or cardiovascular disease (as defined above) was excluded by a detailed medical history A sufficient number of controls were collected t allow statistical analysis. T ensure sufficient numbers for blood lipid estimations, nine persons were added to the control group who did not participate in the BP study though it had been confirmed that they had no family history of hypertension

METHODS

BP was recorded indirectly using a 13 cm cuff on the right arm connected to a mercury column Two measurements were made for each subject 1) directly on arrival (supine) and) after 10 min rest (supine) The disappearance of the fifth sound of Korotkow was taken as the diastolic BP level. Height and weight were meas- red Physical examination included auscultation of the heart and palpation of the femoral arteries. Blood was drawn for cholesterol triglycerides, creatinine as ell as Hct and ESR. Urine was analysed for protein, glu cose and cells. An ECG was recorded from which the pulse rate was read except in 16 controls for whom ECG was lacking du to various reasons. In those cases the pulse rate was registered by digital palpation of the radial artery Each examination was carried out by one of the authors and was performed in the same examination room with the same instruments. As a rule the examination took place in the morning with the subject

Table I *Age distribution*

		Age group			
		<33 y		≥33 y	
	Total	Men	Women	Men	Women
Familial group					
Mean	31 9	24 0	25.8	38 6	40.3
N	84	20	4	19	21
Control group					
Mean	31 1	24.6	26.4	38.8	39 4
N	61	18	15	9	19

Table II *Means of resting supine diastolic pressure (RSDP)*

	Age group		
	<33 y	≥33 y	Total
Familial group			
Men	71 0	87 4	79
Women	73.3	85 7	79.5
Men+women	72.2	86.5	79 4
Control group			
Men	67.5	75.6	71.5
Women	72.3	77 9	75 1
Men+women	69 9	76.7	73 3

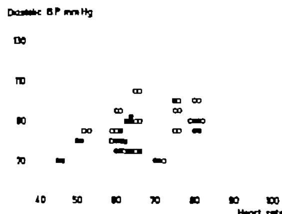


Fig 3 Resting pulse rate versus RSDP in members of families with heavy aggregation of essential hypertension. ■=males <33 years □=males ≥33 years ●=females <33 years, ○=females ≥33 years.

fasting. Exceptions were most of the controls, who were examined later in the day and were not fasting. Cholesterol and triglycerides of the latter were checked on later day in the morning and in fasting state.

From the outset the clinical material was di ided on the basis of age se and family history I addition each group was arbitrarily d vided into two those between 17 and 32 years of age and those between 33 and 49 This division produced similarly sized groups (Table I) The resulting groups served as cells in the un weighted analysis of variance. Testing of hypotheses was done according to *F*-form, and the level of signifi cance was 5% Additional analyses were made using contrasts (the level of significance corrected by F s cher method) and Hotelling T^2-test.

RESULTS

Blood pressure Resting supine diastolic pressure (RSDP) was used in the statistical analysis The

Table III Means of resting supine systolic pres sure (RSSP)

	Age group		
	<33 y	≥33 y	Total
Familial group			
Men	132.0	140 5	136.3
Women	125 6	148 1	136 9
Men+women	128.8	144 3	136.6
Control group			
Men	131 1	132.8	13_0
Women	1_5.3	132 4	128 9
Men+women	128.	13? 6	130 4

mean RSDP was found to be significantly higher in the groups with positive family history than in the control groups ($p<0.05$) (Table II Figs 1–3). No significant difference could be attributed to sex in the different groups BP as expected was found to be higher in the older groups (controls and familial groups taken together) ($p<0.05$) When variance was analysed a nearly significantly higher BP ($F=3$ 7) was found in the older groups with a positive family history than in the corresponding control groups. This was confirmed by additional analyses using contrasts No such difference could however be demonstrated between the younger groups

The means of the resting supine systolic pres sure (RSSP) showed the same pattern among the groups as the RSDP (Table III) Howe er the variance was greater within the groups.

The distribution of the systolic and diastolic BP in the total material is demonstrated in Figs 4 (younger groups) and 5 (older groups) By drawing a line cutting the abscissa between 90 and 95 mmHg diastolic pressure in Fig. 5 for instance the difference between the familial and the control groups is easily demonstrable To the right of this line 1 familial cases are situated (30% of the older familial group) as compared to a single case of the control group (4% of the older control group) By drawing a line cutting the ordinate horizontally between 150 and 155 mmHg systolic pressure an equivalent situation is found concerning the systolic pressures abo e this line (3 and 11% respectively) The differences between the casual supine diastolic pressure (CSDP) and the RSDP (ΔCSDP–RSDP) on the one hand, and the casual supine systolic pressure (CSSP) and the RSSP (ΔCSSP–RSSP) on the other were cal culated. ΔCSDP–RSDP was -4 mmHg in the familial groups nd 1 3 mmHg in the controls. The corresponding figures for ΔCSSP–RSSP were 5 1 and 3 0 mmHg respectively These numerical dif ferences are not statistically significant.

The resting pulse rate was surprisingly lower in the familial groups than in the in controls and the difference was statistically significant ($p<0.005$) (Table IV) The pulse rate was significantly higher in women than in men irrespecti e of age ($p<0.005$). The pulse rates obtained by digital palpa tion did not differ significantly from those obtained from ECGs Plotting the RSDP ersus the resting pulse rate in the familial groups gave a pat

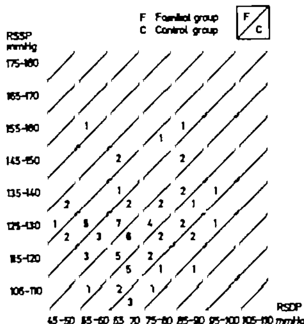


Fig 4 Distribution of RSSP and RSDP in younger age groups

tern with no individuals of the younger age groups situated in the low pulse–high pressure quadrant (Fig. 3) Calculation of the correlation coefficient in the different age and sex groups showed a significant positive correlation between RSDP and pulse rate in the familial group of young -s only ($r=0.63$ $p<0.005$). The same was for RSSP in relation to resting pulse rate in familial group of young females ($r=0.55$ $p<0.01$) No such correlations were found in controls The correlation between RSDP and resting pulse rate was not significantly changed on successive testing of still younger (and smaller) groups of males belonging to the families However below the age of 23 the number of individuals was too small to reach significance

Blood lipids The means of the serum triglycerides and serum cholesterol were higher in the older than in the younger groups and the difference was statistically significant ($p<0.05$) (Table V) There was no difference in serum triglyceride level between the familial and the control groups. However such a difference was demonstrable concerning *serum cholesterol* which was significantly higher in the familial groups ($p<0.05$).

Anthropometric data Length and weight did not differ between the familial and the control groups ($p>0.25$) (Table VI).

Table IV *Means of resting pulse rate*

	Age group		
	<33 y	≥33 y	Total
Familial group			
Men	64.5	6_1	63 4
Women	71.5	70.4	71.0
Control group			
Men	65 9	71 4	68.0
Women	76.9	75 1	76 0

DISCUSSION

The finding of a higher BP in the familial groups than in controls is in accordance with earlier investigations of family members of patients with assured hypertension regardless of its cause (4, 8 17) and with similar studies in which the hypertensive index cases, as in this study had first been investigated to rule out secondary hypertension (7 14).

The rise of BP with age as demonstrated in this work is common medical knowledge and is

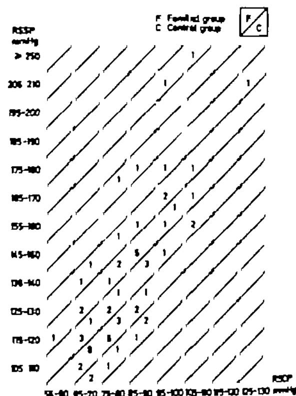


Fig 5 Distribution of RSSP and RSDP in older age groups.

Table V *Means of serum triglycerides and serum cholesterol*

	Age group		
	<33 y	≥33 y	Total
Serum triglycerides (mmol/l)			
Familial group			
Men	1.30	1.45	1.38
Women	1.14	1.28	1.21
Men+women	1.22	1.37	1.29
Control group			
Men	1.05	1.57	1.28
Women	0.94	1.35	1.14
Men+women	0.99	1.43	1.[illegible]1
Serum cholesterol (mg/100 ml)			
Familial group			
Men	222.4	[illegible]38.3	230.3
Women	10[illegible]	[illegible]41.4	225.8
Men+women	216.3	239.8	228.1
Control group			
Men	188.6	223.9	[illegible]06.3
Women	184.4	[illegible]42.6	[illegible]13.5
Men+women	186.5	233.3	209.9

well documented in the literature e.g. in the large population study by Hamilton et al (8).

The resemblance of BP within families in different age groups even in young people and small children, has been demonstrated before (8 [illegible]0). Smirk (17) found in a controlled study of the BP level of 519 first degree relatives of propositi with "substantial hypertension" that the casual and basal BP are higher in the relatives especially above the age of 40. However Smirk was concerned with hypertension regardless of its cause be accepted any first degree relative and did not, as in our study, pick out those with additional cases of hypertensive disease in the family. The finding of a cut-off point of age above which there is a considerable increase of the BP level in members of families with a heavy aggregation of essential hypertension has its practical implications in the important field of preventive medicine. We have found no earlier study of this specific kind in the literature. Thus on the basis of this investigation it seems reasonable to start screening of members of such high risk families at the age of 30–35.

Table VI. *Anthropometric data*

	Age group		
	<33 y	≥33 y	Total
Body weight (kg)			
Familial group			
Men	74.42	76.35	75.41
Women	63.13	63.65	63.37
Control group			
Men	72.29	77.85	74.70
Women	59.00	64.58	61.59
Body length (cm)			
Familial group			
Men	179.8	175.5	177.6
Women	165.7	16[illegible]3	164.1
Control group			
Men	180.1	179.3	179.8
Women	168.7	165.0	166.9

Smirk found in an early study (16) that the difference between the casual and basal BP was greater among hypertensive individuals than among normal subjects. However he did not find this to be so in his study of relatives of hypertensives referred to above (17). The difference in our study between casual and resting BP is numerically greater in the familial groups than in controls but not sufficiently for statistical significance.

The somewhat unexpected finding of lower resting pulse rates in the familial groups compared to controls is probably attributable to the fact that all familial cases were examined in the early morning, and this was the case for only a minor part of the controls the majority of whom were seen in the afternoon. Richardson et al found in 1964 that the pulse rate rises during the day both in normal and hypertensive subjects (15). Our finding of higher pulse rates in women than in men is in accordance with earlier investigations (3).

The finding of a significant positive correlation between BP and pulse rate in the younger familial groups but in no other group is consistent with the concept of essential hypertension starting as a high output pressure with normal peripheral resistance later merging into a normal or low output pressure with raised peripheral resistance (9).

Our finding of higher blood lipid levels in the older than in the younger groups is in accordance with earlier investigations concerning both serum triglycerides and serum cholesterol (6). The reason for the unexpected finding of higher serum cholesterol in the familial groups than in controls cannot be sought in the variation of body fat mass (1) because length and weight are on the same level. In addition such a cause would reasonably affect

the serum triglycerides to a greater extent (1) As no adequate methodological control was practised in the laboratory consulted at the start of the study and the bulk of the familial cases were examined before the mass of controls it cannot be excluded that there may have been some methodological drift during the course of the investigation.

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DOPAMINE β-HYDROXYLASE IN HYPERTENSION

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Abstract. Dopamine-β-hydroxylase (DBH), the enzyme responsible for the biosynthesis of noradrenaline from dopamine, has been assayed in the blood plasma of 53 hypertensive patients. The results were compared with those from a control group of 93 adults. The plasma DBH activity varied within a wide range in both hypertensives and controls. There was no difference between the average DBH activity nor any significant relation between the resting recumbent systolic or diastolic BP and the plasma DBH activity. In a subsample of the material consisting of 11 patients and controls matched for age and sex, the DBH activity was studied at 3-hour intervals during 12 hours in an attempt to estimate the variation of each individual. No significant difference was found between the two groups.

The role of the sympathetic nervous system in the pathogenesis of primary hypertension is not known. Recently some reports on the catecholamine level in serum from hypertensive patients have been published. De Quattro and Chan (7) described an increased level in patients with primary hypertension. Furthermore, Louis et al. (4) found a highly significant ($p<0.001$) relation between the resting recumbent BP and the plasma level of noradrenaline.

The enzyme dopamine-β-hydroxylase (DBH) which is responsible for the synthesis of noradrenaline from dopamine is released together with noradrenaline from the sympathetic nerve terminals and can be detected in the blood (11). The blood level of DBH may then be a measure of the activity of the sympathetic nervous system (11). If this system is in hyperactivity in the state of hypertension it might be reflected by an elevated level of DBH in the blood circulation. In the present study we have investigated the level of plasma DBH activity in patients with primary hypertension, the relationship between the BP level and DBH, and finally the daytime variability.

MATERIAL A

Seven female and 46 male patients with hypertension were studied (Table I). The mean age was 45.7±1.4 years (range 17–71). They were selected on a first available basis and mainly collected during a time span of 6 months. The patients had primary hypertension, except three who had renovascular causes of their hypertension. They had never received any antihypertensive drug.

Plasma DBH was sampled in the morning with the patients fasting. Immediately before the plasma sampling BP was measured by the auscultatory method after 10 min rest. This material was evaluated with regard to the level of plasma DBH and whether any relationship existed between DBH and resting BP.

The control group consisted of 93 apparently healthy adults of the hospital staff and blood donors of both sexes with the same age distribution as the hypertensives.

MATERIAL B

Eleven patients (Table I) with primary hypertension were studied. The first DBH was taken under similar conditions to those in material A. The other specimens were taken at 3-hour intervals over 12 hours (a total of 5 specimens from each patient). The patients were on regular daytime hospital activities. None had antihypertensive therapy. In the calculations the lowest value of the five specimens was determined as 100 (arbitrary units). The different values were then recalculated and could be added together for usual statistical analysis.

The control group was obtained from the Departments of Medicine and Surgery, University Hospital, Uppsala. The controls were matched with regard to age and sex, and the only conditions for acceptance were normotension and lack of medication. The group variability was estimated as in material B.

METHOD

Venous blood was collected into heparinized plastic tubes. Within half an hour the blood was centrifuged and the plasma separated and stored at −20°C until assayed.

Table I *Data on the patients*

	Material A	Material B
No. of pats.	53 (♂/♀ 46/7)	11 (♂/♀ 7/4)
Age (y.)	45.7±1.4	44±3.9
Heart volume (ml/m² BSA)	423±.3 (300–750)	438±39 (300–750)
Serum creatinine (mg/100 ml)	1.0±0.06 (0.7–1.8)	0.9±0.06 (0.7–1.2)
No. of pats.		
LVH[a] (ECG)	5	4
FH[b]		
0–1	20	5
I	14	
	19	4

ECG criteria according to Goldman. Keith-Wagener classification.

Table II *Results of the determination of DBH activity*

		DBH activity (nmol/ml/20 min)				
					Quartile	
	No. of cases	Mean	Range	Median	Lower	Upper
Hypertensives (A)	53	214±18	5–569	186	119	287
Control	93	225±16	0–715	193	103	319

DBH was determined enzymatically according to the method of Molinoff et al. (6) as modified by Weinshilboum et al. (13). β-phenylethylamine was used as the substrate and the pH of DBH was kept at 5.2, the optimal pH for this enzyme. The incubation mixture consisted of 200 μl blood plasma diluted 1:50 with a 5× M solution of CuSO in distilled water, 25 μl 8 M ascorbic acid, pH 6, 25 μl 0.5 M sodium fumarate, pH 6, 20 μl 0.006 M pargyline, 1500 U catalase in 10 μl distilled water, 10 μl 0.03 M β-phenylethylamine and 10 μl 1.0 M sodium acetate buffer, pH 4.9.

After 20 min incubation at 37°C the DBH reaction was stopped and the enzymatic determination of the β-phenylethanolamine formed was started by addition to each tube of 100 μl of a fresh mixture of 10 μl purified PNMT (phenylethanolamine-N-methyltransferase), 10 μl S-adenosylmethionine-methyl-¹⁴C (1 nmol) and 80 μl 1.0 M Tris buffer, pH 8.6. After 30 min incubation the reaction was stopped by addition of 0.5 ml 0.5 M borate buffer, pH 10. The N-methyl-¹⁴C phenylethanolamine formed was extracted by vigorous shaking into 6 ml toluene containing 3% isoamyl alcohol.

Table III *Variability within material B and controls (arbitrary units)*

	No. of cases	Mean	Range	S.E.M.
Hypertensives (B)	11	123 ns	(100)–240	4.0
Controls	11	115 ns	(100)–157	1.9

ns = not significant.

After centrifugation 4 ml of the organic layer were transferred to counting vials containing 10 ml scintillation liquid and the radioactivity was determined. Blanks consisting of diluted plasma heated to 95°C for 5 min and internal standards (100 ng phenylethanolamine hydrochloride) were run through the whole reaction procedure. The internal standards were used for the calculation of the DBH activity, expressed in nmol of β-phenylethanolamine formed from 1 ml plasma during 20 min incubation. The DBH activity was determined in triplicates and the mean value is reported. A plasma sample was used as an external standard in order to make the results from different assays comparable.

RESULTS

In the control group as well as in the hypertensives the plasma DBH activity varied within a very wide range. The activity of one patient was less than 10 nmol/ml/20 min. Six of the controls had no detectable DBH activity. (Among 91 hypertensives so far we have found none without measurable DBH activity compared to 6 of 93 controls.) The average DBH activity in material A was 214±18 and in the control group 225±16 nmol/ml/20 min. The difference between the means was not significant (Table II).

The difference in variability within material B and within the control group did not reach significance (Table III). Finally, no significant relation

was found in material A between plasma DBH activity and the resting recumbent systolic BP ($r=-0.156$, $y=178x-0.020$) or the diastolic BP ($r=0.052$, $y=111x+0.004$)

DISCUSSION

Observations in several laboratories have shown that there is a great individual variation of the plasma DBH activity (2, 8 13 14) Alterations in DBH activity induced by disease may therefore be lost in this wide range of activity The variation of the plasma DBH seems not to be related to differences in sympathetic nerve activity since there are apparently healthy persons with no detectable DBH activity in their blood In a recent study of the plasma DBH in monozygotic and dizygotic twin pairs it was observed that the level of the plasma DBH is genetically controlled (9) The same conclusion was independently made by Weinshilboum et al. (13) from the results of a sibling study Other factors than the sympathetic nerve activity may accordingly determine the level of the plasma DBH activity This hypothesis does not preclude that the sympathetic nerves and the adrenal medulla are the main sources of the plasma DBH In order to unmask the influence of the wide individual variation it would be preferable to use healthy siblings as controls in studies of the DBH activity in disorders (5)

In a preliminary study (14) we reported an elevated DBH activity in hypertension. This result is not verified by the present extended study and was apparently due to an insufficient material Horwitz et al (3) were also unable to observe any changes in serum DBH activity in a study of hypertensives These observations do not necessarily mean that plasma DBH activity does not rise in hypertension in the individual but they indicate that the elevation is not large enough to be perceptible in the wide individual variation. An observation that indicates that this really may be the fact is the lack of subjects without detectable DBH activity among the hypertensive material whereas six persons were without plasma DBH activity in the control material

The conclusion drawn from the present study is that there is no difference in plasma DBH activity in a random sample of hypertensives compared to controls. The reason for this negative finding may be that other factors than the activity of the sympathetic nervous system dominate the regulation of the plasma DBH level A study of hypertensive patients with their healthy siblings as controls can perhaps elucidate whether the plasma DBH like plasma noradrenaline (1 4 7) is elevated in hypertension.

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THE EFFECT OF POTASSIUM CHLORIDE AND SPIRONOLACTONE ON THIAZIDE INDUCED POTASSIUM DEPLETION IN PATIENTS WITH ESSENTIAL HYPERTENSION

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Abstract. Total exchangeable potassium (K_E), red blood cell potassium (K_{RBC}), serum potassium (K_S) and blood pressure (BP) have been determined in 34 patients with essential hypertension after not less than 4 weeks therapy with hydrochlorothiazide 75 mg daily K_E was significantly lower than the levels found in untreated patients with essential hypertension, determined by the same method and in the same laboratory K_{RBC} and K_S were also significantly lower than the values in normal individuals. The patients were then divided at random int two groups, one group receiving potassium chloride supplement of 60 mEq/d, the other spironolactone supplement of 100 mg/d. After 5 weeks of combined therapy the above parameters were again determined. Both groups presented a significant rise of K_E, K_{RBC} and K_S. The rise of K_S in the spironolactone group was significantly higher than that found in the potassium chloride group but the increase of K_E and K_{RBC} in the two groups did not differ significantly Spironolactone caused a significant reduction in systolic as well as diastolic BP while potassium supplement did not influence the BP

With a few exceptions (1 14) numerous studies have shown that continuous diuretic therapy will produce hypokalaemia and a reduction in total body potassium (2, 11 12 17 20) This is supported by the finding in muscular tissue of reduced potassium levels following diuretic therapy (3).

To counteract this potassium deficiency supplements of potassium chloride spironolactone or another potassium-sparing diuretic may be added. Such therapy will cause the serum potassium to rise but it remains to be clarified whether this is accompanied by an increase in the total body potassium. Results of studies on this problem are contradictory

The object of the present study was to examine the effect of potassium chloride and spironolactone supplements on total exchangeable potassium (K_E) red blood cell potassium (K_{RBC}) and serum potassium (K_S) n patients with arterial hypertension on long-term hydrochlorothiazide therapy

MATERIAL AND METHODS

Studies were performed on 34 patients, 25 males and 9 females, with essential hypertension of slight to medium severity All patients had been investigated to exclude known causes of hypertension, the programme including determination of serum electrolytes, urinary excretion of catecholamines, urography and isotope renography None of the patients had present or previous cardiac failure, and in all of them normal renal function was found assessed on the basis of serum creatinine and creatinine clearance. Twenty of the patients had received thiazide for more than one year on an verage 4 years prior to the study The remaining patients had been treated for only few months or had started treatment at the commencement of the study

The patients were given 25 mg hydrochlorothiazide three times daily for at least 4 weeks. No other antihypertensive therapy was given, and any previous supplement of potassium or potassium-sparing diuretics was withdrawn. K_E K_{RBC} and K_S were then determined. BP was measured on the same day All investigations were made in the Outpatient Clinic while the patients were fasting. No other dietary restrictions were made, and the patients were instructed not to change their normal diet. The aim of the study was explained to the patients and all agreed to participate.

Following the determination of the initial values, the patients were divided at random into two groups. One group received supplement of 20 mEq potassium chloride three times daily and the other 25 mg spironolactone four times daily After 5 weeks of combined

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Table I. K_E *in thiazide-treated and untreated (6) hypertensive men and women*

	Thiazide treated	Un-treated	p
Men			
No	25	1	
Mean age (y.)	50	49	
K (mEq/m² BSA)			
Median value	1 41	1 628	<0.05
Interquartile range	1 306–1 5.3	1 366–1 718	
Women			
No.	9	22	
Mean age (y.)	48	54	
K (mEq/m² BSA)			
Median value	1 128	1 387	<0 01
Interquartile range	978–1 156	1 168–1 476	

therapy the bove parameters were again determined. Spironolactone (Aldactone) wa placed at our di posal by G D Searle Ltd

K wa determined accord g to Christensen and Galskov (6). Appro imately 75 μC ⁴²K were injected i. t 8 a.m. After 24 hours equilibration the pecific activity was determ ned in a 1-hour spot rine sample obtained between the 24th and 25th hour after the injection ⁴²K was counted in liquid sample Ge ger Möller counter K wa then calculated according to the ordinary isotope dilution principle

K_{RBC} w s determined as described in another paper (3). This method i based on determination of whole-ood potassium (K_B), K and haematocrit (H). K_{RBC} ll then be

$$K_{RBC} = \frac{100}{H}(K \quad - K_B) + K_s$$

expressed n mEq/l packed red cells

1 45 controls the normal range of K_{RBC} expressed as the 2.5 and 97.5% percentiles, wa 94 0–105 7 mEq/l (median value 98 4 =45). The results found in males and females were identical. K l the same 45 controls, expressed as the 2.5 and 97.5% percentiles was 3 6–4 8 mEq/l (median value 4 3)

BP was measured with an armlet and a mercury manometer at 8 a.m after at least 10 min in supine position The values stated are the averages of three consecutive measurements.

One patient had to be excluded after weeks combined therapy with thiazide and pironolactone because of menometrorrhagia.

Statistical calculation were made by employing the Student's *t*-test for paired observations and the Mann-Whitney rank-sum test for unpaired data. When unpaired data were compared they were expressed by median value and interquartile range (central 50% range). In order to evaluate the reproducibility of the method for measuring K_E two initial determinations were made in 10 patients at intervals of 1 week, the S.D of these being 163 mEq

RESULTS

After at least 4 weeks therapy with hydrochlorothiazide alone (Table I) the total K_E in males and females indicated by median value and interquartile range was 1412 mEq/m² BSA (1 306–1 523) and 1 128 mEq/m² BSA (978–1 156) respectively These values are significantly lower than those found by Christensen and Galskov (6) in males and females with untreated hypertension the age distribution of their patients being similar to that of the present series K_{RBC} levels were also lower than those found in normal subjects median value 9? 9 mEq/l (interquartile range 90 4–94 4) ($p<0$ 01). As stated above the normal range of K_{RBC} was 94 0–105 7 mEq/l the median value being 98 4 mEq/l K was also low median value 3 2 mEq/l (interquartile range 2.9–3 4)

Table II. *Change in* K_E, K_{RBC}, K_s *and BP during combined treatment ith hydrochlorothiazide and potassium chloride for 5 weeks in 17 patients*

	K (mEq/m² BSA)	K_{RBC} (mEq/l packed cell)	K_s (mEq/l)	BP (mmHg) Systolic	Diastolic
Thiazid (75 mg/d)					
Mean	1 364	92.6	3 2	153	97
S D	±217	±2.4	±0.3	±19	± 7
Thiazide+potassium chloride (60 mEq/d)					
Mean change	+153	+2.6	+0 35	+ 4	0
S.D of change	±148	±3 0	±0 21	±13	±10
t	4 2575	3 6032	6.6475	1 4543	0.0733
p	<0.001	<0 005	<0.001	n s	n s.

Table III Changes I K K$_{RBC}$ K and BP during combined treatment with hydrochlorothiazide and spironolactone for 5 eeks in 16 patients

	K_E (mEq/m² BSA)	K_{RBC} (mEq/l packed cells)	K_s (mEq/l)	BP (mmHg) Systolic	Diastolic
Thiazide (75 mg/d)					
Mean	1 3‿	92.6	3 1	148	93
S.D	±207	±3.3	±0.5	±14	±7
Thiazid + spironolacton (100 mg/d)					
Mean hange	+ 81	+‿7	+0 88	−18	−7
S.D of change	±104	±3 3	±0 33	± 9 6	±8 4
t	3 1080	3.3066	10.5654	7 7390	3 4999
p	<0 01	<0 005	<0.001	<0.001	<0 005

After 5 weeks combined therapy with 75 mg hydrochlorothiazide and 60 mEq potassium chloride daily K_E increased signficantly on an average 11% ($p<0.001$) (Table II) Also K_{RBC} rose significantly by an average of ‿6 mEq/l ($p<0\ 005$) and K_s increased by 0.35 mEq/l ($p<0\ 001$) BP was not influenced by the potassium supplement.

Patients who received spironolactone supple ment 100 mg daily presented a significant increase in K on an average 6% ($p<0\ 005$) (Table III) K_{RBC} increased on an average by ‿7 mEq/l ($p<0\ 005$) There was a considerable increase in K_s 0.88 mEq/l on an average ($p<0\ 001$) Both systolic and diastolic BP were signifi cantly reduced 18 ($p<0\ 001$) and 7 mmHg ($p<0\ 005$) respectively

With the doses administered in the present study the increase i K_s during spironolactone therapy was considerably higher than the increase obtained by potassium supplement ($p<0\ 01$) Although the rise in K_E might seem higher with potassium supplement, the difference however is not significant ($p>0\ 1$). The changes in K_{RBC} did not differ in the two groups

There was a significant though not particularly pronounced correlation between K_E/m^2 and K after thiazide therapy ($r=0.3490$ $p<0\ 05$ $n=34$).

The interrelation between the changes in K_{RBC} and K_E was not particularly pronounced but will be described in another paper (13)

Very few side-effects were observed during the examination period. Only one patient developed symptoms of hypokalaemia such as pronounced fatigue and muscle weakness These symptoms occurred when only hydrochlorothiazide was given, and K_s was then 1 9 mEq/l Three females suffered from postmenopausal menstruation-like bleeding during spironolactone therapy and as previously mentioned one patient was excluded for that reason.

DISCUSSION

Following thiazide therapy without potassium supplement or administration of potassium-sparing diuretics for at least 4 weeks the K was significantly lower than the levels found in patients with untreated essential hypertension, determined by the same method and n the same laboratory (6) As stated in the introduction this is with a few exceptions consistent with findings made in numerous other studies. The females included in the present study seemed to be more potassium depleted than the males It appears from Table I that total potassium in males and females treated with thiazide was 90% and 80% respectively of K_E in untrated subjects Correspondingly K_s as shown by median value and interquartile range was 3 3 mEq/l (3 1–3.5) and 3 0 mEq/l (2.7–3 2) respectively ($p<0.01$). The average weight of males and females being 83 0 and 63 1 kg respec tively the most probable explanation is that the females received relative over-dosage" as compared with the males Also the K_{RBC} levels were lower than those found in normal subjects and this supports the theory that there must be an intracellular deficiency This is in accordance with the findings of Boyd (4)

With a potassium supplement

the total potassium increased by about 11%. This change was almost of the same order of magnitude as the deficiency during thiazide therapy found by other authors (2, 11 12 17). The increase in K_{RBC} showed also that potassium was retained in the cells. The change in K_s was slight but significant, and in accordance with that found in other studies (6 11) although the increases found in several investigations were not significant (1 14 15).

The change in total potassium observed was consistent with the findings made in several studies (11 15) but differed from others (1 14). Investigations of patients with chronic heart diseases who were treated with diuretics have also presented contradictory results (10 20). These discrepancies do not seem to be due to the different methods employed. In studies where K_E was determined by means of ^{42}K (6 10 15) and in investigations determining total potassium by whole-body counting of ^{40}K (1 11 14) both increased total potassium and unchanged levels have been found. In the studies where potassium depletion occurred after thiazide treatment, and where potassium supplement was given a significant rise in total potassium was found this finding accords with the present study (11 15 20). On other hand investigators who did not observe in the total potassium during treatment diuretics found no changes following potassium supplement either (1 6 10, 14). Possibly the contradictory findings are partly attributable to dosage of diuretics and potassium chloride dietary differences may also exert an influence (2). The potassium supplement dosage given during the present study was higher than that used in other investigations.

Spironolactone supplement increased K_E significantly about 6%. This was consistent with the findings in other studies (6 15) and it has also been proved that spironolactone produces an increase in K_E in patients with primary hyperaldosteronism (5). In a study in which patients with essential hypertension were given only spironolactone, a significant reduction in K_E was found but also an increase in K_s (21) apparently these patients were not potassium-depleted. The increase in K_{RBC} found during the present study supports the above mentioned results.

The increase in K_s found in the spironolactone group was considerable and higher than that found in the potassium chloride group. On the other hand the change in K_E was not markedly different in the two groups. Spironolactone is a specific aldosterone antagonist and inhibits the renal potassium excretion resulting from aldosterone. The results could also indicate that spironolactone exerts an influence on extrarenal cell membranes resulting in a changed distribution of potassium between the extracellular and intracellular compartments, although there are no definite indications that aldosterone and aldosterone antagonists influence other than epithelial surfaces (7 8 16). However this does not exclude the possibility that spironolactone may possess important pharmacological properties independent of aldosterone. Studies on isolated carcinoma cells and human erythrocytes have shown that spironolactone in the absence of aldosterone increases intracellular sodium and decreases potassium (7). Similarly the sodium transport across isolated frog skin is inhibited by spironolactone in the presence as well as absence of aldosterone (8 16).

Whereas BP was not influenced by potassium supplement, a moderate but significant fall was observed both in the systolic and the diastolic BP during spironolactone therapy even though the BP was well controlled when hydrochlorothiazide alone was given. Spironolactone exerts its most pronounced hypotensive effect in patients with low or hyporesponsive plasma renin activity i.e. patients with primary hyperaldosteronism or other mineralocorticoid excess and 20–30% of patients with essential hypertension (9 18). Practically all such patients will be normotensive when treated solely with high doses of spironolactone 400 mg daily (5 9 18). However as proved by the present study and by other investigations (9 18 22) lower doses of spironolactone exert a moderate more non-specific hypotensive effect, which can be compared to that of thiazides, and which is additive when combined with other diuretics (22).

It has often been maintained that serum potassium is a poor expression of the potassium balance. However a significant although not pronounced positive correlation between K_E/m^2 and K_s following thiazide therapy was found. Edmonds and Jasani (11) also found such a correlation.

It is concluded that, in patients with potassium depletion resulting from treatment with diuretics

it is possible to increase both the extracellular and the intracellular potassium by adding potassium chloride or spironolactone. On the other hand it is difficult to draw any definite conclusions as to the necessity of such supplement. It is agreed that, under certain circumstances, it is important to avoid potassium depletion, e.g., in patients with liver cirrhosis or diabetes mellitus and in patients on digitalis. Whether it is necessary as a routine to give a potassium supplement or a potassium-sparing diuretic to hypertensive patients on thiazide therapy cannot, however, be considered to be clarified. In such patients, symptoms which can with certainty be associated with hypokalaemia are extremely rare (11, 12). It is well known, however, that severe potassium deficiency may result in damage to renal tissue and the myocardium, disturbances in the tubular function of the kidneys and in a great many enzymatic reactions, although studies in animals and man could indicate that such damage is not observed until the deficiency is about 20–40 % of the total potassium (19). The reduction in total potassium which is commonly seen during long-term diuretic therapy in patients with arterial hypertension without cardiac complications ranges between 8 and 14 % (2, 11, 12). Hence Healy et al. (17) were unable to find changes in the tubular function of the kidneys or in the carbohydrate metabolism. In the present study the females receiving thiazide treatment had an average K_e of 3.0 mEq/l (interquartile range 2.7–3.–) and a reduction in total potassium of about 20 %. Consequently such potassium deficiencies should be corrected. Whether one drug should be preferred to another during treatment depends on the effect desired. Potassium chloride is at least as effective as spironolactone for increasing total potassium, but if a severe hypokalaemia is to be corrected, spironolactone is to be preferred. Moreover, it has an additive diuretic and hypotensive effect.

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HEMODYNAMIC EFFECTS OF ACUTE AND PROLONGED β-ADRENERGIC BLOCKADE IN ESSENTIAL HYPERTENSION

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Abstract Hemodynamic studies have been performed in 15 male patients with mild to moderately severe essential hypertension. Studies were made after four weeks of placebo treatment and again after acute i.v. administration of propranolol (0.22 mg/kg) and after four weeks of oral propranolol therapy (160–320 mg daily). After acute β-adrenergic blockade a significant reduction of cardiac index (QI) occurred, while total peripheral resistance (TPR) increased significantly resulting in unaltered BP After four weeks of oral propranolol the QI remained significantly reduced, but TPR was readjusted towards the initial level, resulting in reduction of BP in most patients. Major reductions of stimulated plasma renin activity were seen, but these were not correlated to the changes of BP QI or TPR. Studies of the baroreceptor reflex by means of administration of angiotensin suggested a change towards increased sensitivity although this change did not achieve statistical significance.

Antihypertensive effect of propranolol has been reported in several studies (6 8 14 15 20 23). As most forms of established hypertension are characterized by increased total peripheral vascular resistance (TPR) and normal cardiac output (Q) (5 11 17) the antihypertensive properties of propranolol have been hard to explain in view of its predominantly cardiac effects Several suggestions have been offered as tentative explanations e.g. resetting of baroreceptors (15) central nervous effects (6) adaptation of vascular resistance in response to chronic reduction of Q (20) and local anesthetic effects on vasoconstrictor fibers (4) Lately propranolol-induced reduction of plasma renin activity (PRA) in hypertensive patients has been demonstrated (9) which may be of importance in the hypotensive mechanism especially in conditions with high plasma renin. As the effects of acute and chronic administration of propranolol are different particularly in regard to the effect on BP (16 20 1) further confusion has resulted

For this reason the present study was designed with the intention of studying the antihypertensive effect of propranolol, particularly the hemodynamic alterations resulting from acute and chronic β-adrenergic blockade We were interested to see whether TPR would readjust in the presence of continuously reduced Q In addition, special interest was directed to the study of baroreceptor sensitivity and whether a change would take place Finally PRA was studied with the intention of exploring connections between changes of renin and changes of peripheral vascular resistance.

MATERIAL AND METHODS

Fifteen male patients with mild to moderate essential hypertension were studied. Their average age was 44 years (range 26–66) The average known duration of hypertension was 6.5 years (range 0–17). Before entering the study all patients but two were receiving antihypertensive therapy usually consisting of α-methyldopa and/or diuretics.

Secondary causes of hypertension were excluded in all patients by thorough clinical examination, rapid sequence pyelograms and laboratory studies (serum electrolytes, PRA and urinary excretion of aldosterone, epinephrine and norepinephrine). In addition ECG chest X-ray urine microscopy serum creatinine serum lipids and liver function were checked repeatedly during the study

All patients were told of the investigational nature of the study and ga their informed consent to participation in it.

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Pla of study

After stopping all previous therapy the patients were given placebo treatment in a single blind fashion on an outpatient basis. Placebo treatment continued for four weeks after which the patients were hospitalized for baseline studies including the first hemodynamic study They were discharged after four days and were put on oral propranolol 40 mg four times daily as the sole antihypertensive treatment. After two weeks this dosage was increased to 80 mg four times daily unless a diastolic BP reduction of 15 mmHg or more was observed. Treatment with propranolol was continued for another two-week period The average daily propranolol dosage during weeks 3 and 4 was 250 mg. After four weeks of oral propranolol treatment a repeated hemodynamic study was performed.

Hemodynamic methods

The hemodynamic studies were performed in the morning with the patients fasting

Systemic arterial pressure was measured in the brachial artery by a Statham strain gauge receptor (P 23 G) Mean arterial pressure (MAP) was calculated as the diastolic BP+1/3 of the pulse pressure.

Q was determined by the indicator dilution technique (12) using a Gilford densitometer and Indocyanine green (Cardio-Green)

Respiratory movements were monitored continuously by means of bellows attached around the chest and connected to a strain gauge by a rubber tube. The standard leads of the ECG were connected and lead I ᵕas recorded continuously on Gilson polygraph to- ᵕ with intraarterial BP respiration and dye curves.

Stroke volume (SV) was calculated by dividing Q by eart rate (HR) TPR was obtained by dividing MAP by Q and was expressed in arbitrary units (U). Q TPR and SV were corrected for body surface area and the corresponding indices QI TPRI and SVI were derived.

PRA was measured with a radioimmunoassay technique (7) in the initial untreated state during tilt (stimulated) and again in the tilted position after acute and prolonged β-adrenergic blockade

Hemodynamic tudy I

The first resting Q was determined 15 mm after all catheters were placed. The patients were then tilted to a 45° head-up position for 10 min and Q and PRA were determined. Approximately 10 min after resumption and under resting conditions the baroreceptor reflex was assessed. This was done by rapid i. administration of angiotensin (Hypertensin Ciba) usually 1.5 μg, while recording changes in BP and HR. Systolic BP was plotted against the corresponding R–R interval in the ECG using the technique of Bristow et al. (1). Plotting was continued until the pressure peak occurred No data during inspiration were used. Usually 15–30 points could be plotted in this way for each patient. The points were then analysed with a standard correlation test and only if a statistically significant correlation resulted ($p<0.05$) was the BP/R–R slope used for further comparisons.

Propranolol was then administered intravenously 0.05 mg/kg b wt over 2 mm. Q was determined 8 min after the completion of the injection. A additional amount of propranolol 0.05 mg/kg, was then given and Q measurements were repeated after an identical interval. Finally a third injection of 0.12 mg propranolol/kg was given, bringing the total dosage to 0.22 mg/kg. Hemodynamic determinations were then repeated. The 10-min 45° head-up tilt was then repeated, at the end of which hemodynamics were determined.

Hemodynamic study II

The second hemodynamic study was done at the same time of day and nder identical conditions as the first study but following four weeks of oral propranolol treatment. The same studies were repeated with the exception that i.v propranolol was not given and only one 10-min period of tilt with Q and PRA measurements was performed

Statistically significant BP/R–R slopes were obtained at both the first and second hemodynamic study in 7 patients Only data from these 7 patients were used for comparisons (paired *t*-test)

Assessment of β-adrenergic blockade

The degree of β-adrenergic blockade was tested three times during the study by continuous i.v infusion of a β-adrenergic stimulant—isoproterenol—while monitoring HR on the ECG. On each occasion HR was determined before the start of the infusion. Isoproterenol was then given in constant i.v infusion of 3 μg/ml at the rate of 1 ml/ml for 3 min The response to β-adrenergic stimulation was taken as the difference between the final HR and the HR before the start of the infusion.

The first assessment of response to isoproterenol was made after four weeks of oral placebo treatment before any propranolol had been given. The second determination was made after i.v administration of 0.22 mg propranolol/kg, and the final assessment after four weeks of oral propranolol therapy and approximately 2 hours after the last dose.

Plasma propranolol determinations

Plasma propranolol concentration was determined by fluorimetric technique (18) by Ayerst Laboratories, Montreal, Canada. Blood samples were drawn 70 min after the completion of the i v injection of propranolol and again following four weeks of oral treatment, approximately 90 min after the last dose. The plasma was separated by centrifugation and chilled to 4°C before being sent in an isolated tube for analysis.

Statistical methods

Student s *t*-test the *t*-test for paired observations and correlation coefficients were calculated using the Midas (Constat) computer program of the Statistical Research Laboratory at the University of Michigan.

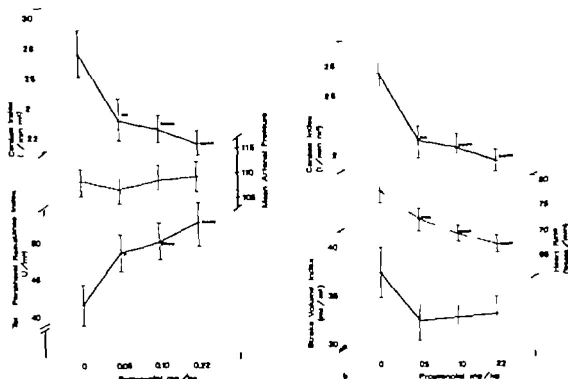


Fig. 1 a and b. Effect of increasing doses of L propranolol (mean ±S.E.M.). The cumulated doses on the *x*-axis were given with 10-min intervals. Statistical comparison (paired *t*-test) is with initial values. * $p<0.05$ ** $p<0.01$ *** $p<0.005$ **** $p<0.001$

RESULTS

Effect of intravenous propranolol

As illustrated in Fig. 1*a* even 0.05 mg/kg of i.v propranolol caused a marked drop of Qi from 2.76 to 2.29 l/min m² ($p<0.01$). TPRi increased from 41.3 to 48.5 U/m² ($p<0.05$) thereby minimizing the effect on MAP which remained virtually unchanged (108.3 vs. 107.2 mmHg, $p>0.25$)

Further administration of propranolol to a total dosage of 0.10 and 0.22 mg/kg, respectively caused further non-significant reductions of Qi and increments of TPRi while MAP remained unchanged (Fig. 1*a*). The overall hemodynamic effects are given in Table I. As illustrated in Fig. 1*b* the reduction of Qi was mainly the effect of decreased HR, while SVi did not change significantly

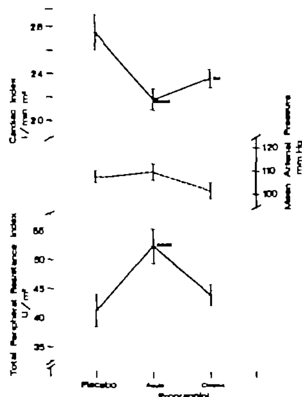
Effect of four weeks of oral propranolol therapy

The effect of four weeks of oral propranolol therapy was similar to the acute effects of i.v propranolol in that Qi remained at a significantly lower level than initially albeit the reduction was

Table I. *Hemodynamic changes at rest*

		Propranolol					
	Placebo	I.v.[a]	Difference[c]	$p<$	Orally[b]	Difference[c]	$p<$
Qi (l/min·m²)	2.76	2.18	0.58	0.001	2.35	0.41	0.01
MAP (mmHg)	108.3	109.9	1.7	n.s.	102.1	6.2	0.05
TPRi (U/m²)	41.3	52.3	11.0	0.005	43.9	–6	n.s.
HR (beats/min)	76.0	67.1	8.9	0.0001	59.6	16.4	0.0001
SVi (ml/m²)	37.3	33.0	4.3	n.s.	40.–	2.9	n.s.

[a] 0.22 mg/kg. [b] For 4 weeks, 160–320 mg/day. [c] Compared to measurement after placebo.



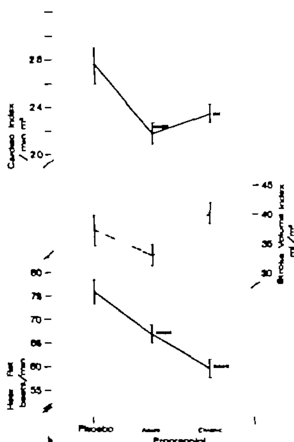


Fig 2a and b Effects of acut i v and chronic oral administration of propranolol (mean ±S.E.M) Statistics as in Fig. 1

ot as pronounced (Table I Fig. 2*a*) The reduction of Qi was mainly an effect of reduced HR, whereas SVi did not change significantly (Fig. 2*b*)

However in contrast to the effect of acute β-adrenergic blockade there now was a reduction of MAP (Fig 2*a*) TPRI was correspondingly less elevated than after acute propranolol and as a matter of fact was not significantly higher than in the untreated state

Analysis of the hemodynamic changes in individual patients revealed that, while 12 patients showed reduced MAP after four weeks of propranolol therapy 3 showed no decrease or even an increased MAP For this reason the 12 re sponders" were compared to the 3 non-responders

Already after acute i.v propranolol there was a significant difference in MAP between the two groups (MAP 105.9 vs. 121 4 mmHg, $p<0\ 05$) (Fig. 3) As expected this difference was even more pronounced after four weeks of oral propranolol (MAP 97 1 vs. 122 2 mmHg, $p<0\ 001$) There were no significant differences between responders and non-responders regarding Qi but after four weeks of oral propranolol responders had significantly lower TPRI (42.3 vs 50.2 U, $p<0\ 05$) (Fig. 3) Differences in HR and SVi were insignificant both after acute and chronic β-adrenergic blockade

Effects of tilt

The hemodynamic effects of tilt in the untreated state after acute i v propranolol and after 4 weeks of oral therapy are presented in Table II Propranolol blocked the HR response to tilt both after acute and chronic administration The well known reduction of Q during tilt was observed also after acute and to a lesser extent after chronic β-adrenergic blockade Finally the chronic β-adrenergic blockade did not interfere with the homeostasis of BP during tilt. Significant increases of MAP were seen in this situation both after placebo and after 4 weeks of oral propranolol. On the other hand a non-significant reduction of MAP occurred after acute i.v propranolol

Effect on plasma renin activity

PRA after tilt in the untreated state after acute

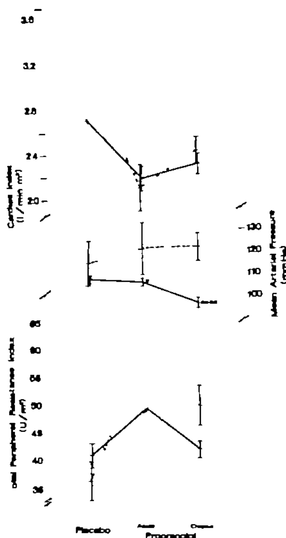


Fig. 3. Effect of acute and chronic oral administration of propranolol (mean ±S.E.M.). Statistical comparison is between responders (—) and non-responders ( ) (Student *t*-test). Probabilities as in Fig. 1.

i.v. propranolol and after 4 weeks of oral therapy are presented in Table III. The effect on PRA of chronic propranolol therapy was striking, causing a reduction of stimulated (tilted) PRA by 85% (from 349 to 39 ng/100 ml/h, $p<0.005$). However, the reduction of PRA was not significantly correlated to the change of MAP, TPRI or Qi either in the recumbent or tilted position (correlation coefficients 0.077–0.104, $p<0.25$). Neither was the change of PRA, expressed in percent, significantly correlated to the percentage change of MAP, TPRI or Qi either recumbent or tilted (correlation coefficients 0.000–0.387, $p>0.10$). There was no difference between responders and non-responders in the reduction of PRA, the three non-responders showing reductions of 81, 90 and 96% respectively, as compared to 85% for the entire group.

Effect on baroreceptor reflex

Only in 7 patients was a statistically significant correlation between systolic BP and R–R interval (BP/R–R slope) obtained after angiotensin injection both in the untreated state and after four weeks of oral propranolol. The average BP/R–R slope in the untreated state was 4.84 and after oral propranolol 7.64. The difference 2.80±1.06 did not quite achieve statistical significance ($p=0.115$) (paired *t*-test).

Effect of isoproterenol

As described before, infusion of isoproterenol was used to assess the degree of β-adrenergic blockade in all patients. In the initial untreated state the HR increased by 39.4 beats/min ($p<0.0001$) in response to the i.v. infusion of 3 μg/min. There was no difference between responders (39.8) and non-responders (38.0) in this respect. After acute

Table II. *Hemodynamic changes during tilt*

	Placebo ($n=15$)				i.v. propranolol[a] ($n=12$)				Oral propranolol[b] ($n=12$)			
	Rest	Tilt	Difference	$p<$	Rest	Tilt	Difference	$p<$	Rest	Tilt	Difference	$p<$
Qi (l/min·m²)	2.76	2.19	0.57	0.005	2.19	1.60	0.59	0.0005	2.44	2.04	0.40	0.005
MAP (mmHg)	108.3	119.6	11.3	0.0005	106.4	98.3	8.1	n.s.	103.4	110.3	6.9	0.005
TPRI (U/m²)	41.3	56.5	15.2	0.0001	50.4	63.8	13.4	0.005	42.6	54.6	10.0	0.0005
HR (beats/min)	76.0	84.9	8.9	0.005	69.5	69.8	0.3	n.s.	57.7	60.6	2.9	0.01
SVI (ml/m²)	37.3	26.4	10.9	0.0001	32.0	22.9	9.1	0.0001	42.7	34.4	8.3	0.0005

[a] 0.22 mg/kg. [b] For 4 weeks, 160–320 mg/d (average 290 mg).

Table III *PRA before and after β-adrenergic blockade*

PRA (ng/100 ml/h) tilt

Placebo	I v propranolol (0.22 mg/kg)	Oral propranolol (160–320 mg/d)
248.7±69.8	204 7±70.1	39 0±10.1
├—— $p<0.01$ —┤		
	$p<0.005$ ——┤	

i.v administration of propranolol and repeated isoproterenol infusion at the rate of 3 μg/min the HR did not increase significantly (1 0 beats/min) Finally following four weeks of oral propranolol the response to isoproterenol infusion was 5.3 beats/min (the increase in responders being 6 1 and in non-responders 2.0 beats/min) indicating that all patients had a significant degree of β-adrenergic blockade

Plasma propranolol determinations

Average plasma propranolol concentration after acute i.v administration of 0 22 mg propranolol/kg was 100.5±6 0 ng/ml and individual variations were small. Following four weeks of oral propranolol treatment, at either 160 or 320 mg daily the average plasma propranolol concentration was 123 1±46 1 ng/ml More than ten-fold variations between individual patients were observed Correlations between the various hemodynamic and PRA changes and plasma propranolol concentrations were not statistically significant either after acute i v or after prolonged oral administration of propranolol. Nor was the response to isoproterenol infusion significantly correlated to plasma propranolol concentration.

DISCUSSION

The effect of propranolol was studied in 15 men with mild to moderately severe essential hypertension. In agreement with previously published results (6 8 14 15 20 23) a BP reduction was seen in most cases following oral treatment.

Hemodynamically it was obvious that the i.v administration of even 0.05 mg propranolol/kg caused a significant reduction of HR and Q while the decrease of SVI did not achieve statistical significance (Figs. 1 and 2). Doubling and quadrupling the dose further decreased HR and Q but the importance of these increments of dosage was minor

Just as important was the finding that BP was not affected by this acute decrease of Q Consequently the calculated TPR had increased to a degree corresponding to the decrement of Q Obviously this is the effect which from a theoretical point of view should be expected from a β-adrenergic blocking agent

After four weeks of oral administration of propranolol the Q and HR were still decreased In contrast to the acute situation however the majority of patients now showed reduced BP with an accompanying reduction of TPR. This finding is in agreement with recent observations of others (20)

It is tempting to speculate about the underlying mechanisms explaining this phenomenon particularly the question of why a chronic reduction of Q would cause a readjustment of TPR in most patients Resetting of baroreceptors has been suggested (15) and our data suggest a change of baroreceptors to a more sensitive setting For a given increase of systolic BP a comparatively greater reduction of HR occurred after treatment. However it should be stressed that we could not demonstrate a significant change of baroreceptor sensitivity possibly due to the small number of patients available for this comparison. Furthermore it should be pointed out that a comparison to baroreceptor sensitivity was not made after acute β-adrenergic blockade However studies of the baroreceptor sensitivity using the same technique as in the present study although revealing some gain of reflex sensitivity after i.v propranolol, showed that atropinization completely abolished the HR response to BP elevation (19). This would indicate that vagal effects are mainly responsible for the baroreceptor-mediated reduction of HR and consequently it would be justified to compare the sensitivity in the untreated state to that after prolonged oral β-adrenergic blockade Preliminary unpublished data of our own reveal that acute i.v administration of propranolol 0 22 mg/kg does not significantly alter the BP/R–R slope

Our tilt results do not support the opinion by Oparil et al (13) that renin plays an important role in the hemodynamic adjustments associated with postural changes The vascular response to tilt after prolonged β-adrenergic blockade was not impaired in spite of marked reduction of PRA. It would therefore seem that renin/angiotensin is not

directly responsible for the hemodynamic alterations during the acute change of posture

The hypothesis that the hypotensive effect of propranolol is secondary to its local anesthetic effect and that in fact the reduction of BP would follow from anesthesia of vasoconstrictor fibers (4) is most interesting. However it is hard to explain the different response to acute and chronic therapy using this explanation. Furthermore studies in man, using the dextroisomer of propranolol which has no β-adrenergic blocking effect but retains the local anesthetic effect, have shown no hypotensi e effect (2_) Tarazi and Dustan (20) were the first to publish results indicating that reduction of BP during propranolol treatment follows from a long-term adaptation of peripheral resistance Obviously our results are in full agreement with their statement, even if at present we are not able to explain the mechanism underlying this adjustment.

The marked reductions of stimulated PRA, and therefore logically also of angiotensin II, could theoretically reduce peripheral vascular resistance However the renin/angiotensin mechanism is not considered to be of etiological importance in mild essential hypertension, and PRA is usually normal or even low in this condition (3 10) Our results are in agreement with such a view as no significant correlation was seen between the change of PRA and the changes of MAP Qt and most importantly TPRt Furthermore the reduction of PRA was equally pronounced in the three patients who showed no decrease of MAP This indicates that reduction of PRA is not the major determinant of the antihypertensive mechanism of propranolol in mild essential hypertension. The lack of correlation between the change of PRA and the change of TPRI indicates that the readjustment of peripheral vascular resistance that takes place during prolonged treatment with propranolol is independent of the change of PRA.

Plasma propranolol concentration was not found to be correlated to either the hemodynamic changes or the effects of i v infusion of isoproterenol which is in agreement with recent observations by others (?4). This could indicate that tissue binding of propranolol is of greater importance than the plasma concentration. Other possibilities are that active metabolites e g 4-hydroxypropranolol, contribute significantly to the degree of β-adrenergic blockade.

Finally the response to isoproterenol infusion failed to single out non-responders Howe er we do not wish to draw any conclusions from this as individual dose–response curves before and after propranolol were not made

CONCLUSIONS

1 Acute β-adrenergic blockade does not cause reduction of BP in patients with essential hypertension. The reduction of Q in this situation is compensated by a corresponding increase of TPR, thereby maintaining BP unaltered

2 After four weeks of β-adrenergic blockade the Qt is still reduced but, due to an adjustment of TPR, the BP is significantly reduced in most patients

3 The alterations of TPR and BP are not directly dependent on the marked reductions of stimulated PRA.

4 An increase of the BP/R–R slope although not statistically significant, indicated that baroreceptors may have been reset towards a greater sensitivity thereby suggesting the mechanism whereby peripheral resistance is adjusted.

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CEREBROVASCULAR LESIONS

Active Antihypertensive Treatment and the Present Situation

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Abstract Active antihypertensive treatment has now been carried out in Sweden for more than 23 years. It has, however been reasonably widespread only for about 15 years. Increasingly efficient heart valve operations have been available for nearly 20 years, with a decline in the number of advanced mitral valvular defects. A downward trend for cerebral vascular lesions from 1950 to 1964 in Göteborg has earlier been noted. A new investigation for the year 1968 shows the same tendency for cerebral haemorrhages and cerebral embolism, but unchanged figures for encephalomalacias. In 1968 as in 1964 considerable number of patients with known hypertension were on inadequate therapy or had been permitted to drop treatment completely

Active antihypertensive treatment in its present meaning has now been available for over 20 years. After 1950 a dramatic change has occurred with a successive decrease of incidence and mortality from cerebrovascular lesions due to hypertension. Earlier publications by Hood et al. (1 2, 3 4 5) have analysed the successive changes of cerebrovascular lesions and active antihypertensive treatment from 1950 onwards to 1964 A considerable reduction both in incidence and mortality has been noted in a population supposed to be actively treated for hypertension.

It was shown that most complications appeared in patients with poor treatment or none at all. Even in 1964 there was a considerable number of patients with hypertension not subjected to active treatment. The question is, then, whether the panorama has changed with time and with improved possibilities for survey diagnosis and treatment and whether there may be still more to gain. In order to answer this question the present study was carried out

MATERIAL AND METHODS

The records from the Departments of Medicine and Neurology at Sahlgren s Hospital in Göteborg, Sweden, were sought for patients admitted in 1968 with acute cerebrovascular lesion. These are the same departments as those in which the analysis quoted above was carried out. Sahlgren Hospital is the only hospital with acute admission wards within the area. As in the quoted material, patients older than 70 years, those belonging to other county council areas and those with recurrent stroke were excluded. To give clearest diagnostic delineation the analyses were also confined to those dying of their cerebrovascular lesion or to cases in which the lesion led to a permanent sequela.

An attempt was made to divide the material, consisting of 111 patients, into the groups cerebral haemorrhage ($n=20$) encephalomalacia ($n=79$) and cerebral embolism ($n=12$).

The diagnosis of cerebral haemorrhage was based upon autopsy cerebral angiography and/or clinical history strongly suggestive of haemorrhage. As complement lumbar puncture was performed. Cerebral embolism was diagnosed upon suggestive clinical history and a reasonable source of embolism In the absence of a rapid onset or of heart disorder causing embolisation the majority were considered to be encephalomalacias.

Diastolic BP on admission was registered Further information about eyegrounds, ECG laboratory findings and other information of interest were noted Of special interest was information concerning previous known hypertension, its treatment, symptoms suggesting hypertension and coexisting disease of importance or complicating conditions. The definition of actively treated hypertension as earlier used by our group requires treatment with at least two drugs or when single drug is used, a diastolic BP below 100 mmHg (6).

As the age distribution is constantly changing, the number of patients has been recalculated corresponding to the number per 10 000 inhabitants at risk in each age group.

RESULTS

The age distribution is shown in Fig. 1 where also the corresponding figures for the years 1949 and 1964 are shown (5) The absolute numbers for the population at risk for each age group in 1968 are also given (7) For all diagnostic groups there is a considerably larger number within the highest age group The corresponding figures for the whole material are given in Fig. 2. It shows that compared with 1964 there is a further decrease for cerebral haemorrhage and embolism while for encephalomalacia the number is unchanged

An analysis of symptoms and signs in patients with haemorrhage and encephalomalacia is shown in Table I About three-quarters of the patients with haemorrhage but not half of those with encephalomalacia had known hypertension prior to the attack However symptoms suggesting hypertension were present in a considerable additional part of the group with encephalomalacia while this was not the case amongst those with haemorrhage A history of positive heredity for cardiovascular disease was obtained in 50% of the encephalomalacias and 32% of the haemorrhages

In the whole material only one patient with eyegrounds corresponding to grade IV and three to grade III according to Keith et al were found

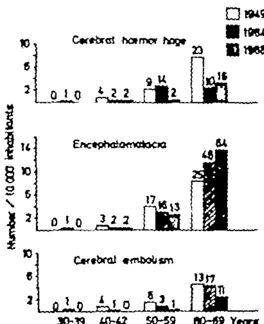


Fig 1 Age distribution of patients with first attack of cerebrovascular lesion leading to permanent disability or death.

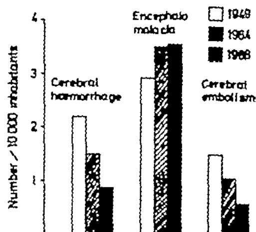


Fig 2 Diagnostic breakdown of first attack of cerebrovascular lesion leading to permanent disability or death.

A survey of other complicating factors on admission in the patients with stroke is given in Table II In the group with cerebral haemorrhage there were no conditions involving a haemorrhagic tendency but three patients presented with other serious complications viz. one with other vascular disease one was diabetic and one chronic alcoholic The only three surviving patients with haemorrhage were the ethylist, who had a normal BP and two patients without complicating factors and a diastolic BP on admission of 120 and 110 mmHg respectively whose antihypertensive treatment had been dropped or inadequate A substantially greater number of complicating factors both of haemorrhagic type (dicoumarol treatment) and non-haemorrhagic type (diabetes known hyperlipidaemia chronic alcoholism angina pectoris claudicatio intermittens uraemia and syphilis) were found amongst the encephalomalacias.

When cholesterol and triglyceride were determined during fasting directly after admission an elevated value was supposed to be significant for hyperlipidaemia For the patients with encephalomalacia, sampling according to those conditions was done for cholesterol in 50% and for triglyceride in 39% Among the latter the levels were elevated (cholesterol ≥300 mg/100 ml triglyceride ≥2.0 mmol/l) in 37 and 23% respectively Manifest diabetes was present in 14% the same figure as obtained in the earlier material by Hood et al (5)

An analysis of the cases of cerebral embolism showed that valvular heart disease with or without arrhythmia was the possible cause in five pa-

Table I *Analysis of symptoms and antihyper tensive treatment prior to cerebrovascular l sion*

	Haemorrhage		Encephalomalacia	
	(n)	(%)	(n)	(%)
Anamnesis not obtained	4	20	7	9
History blank	3	15	29	37
Acute episode( ) earlier			5	6
Continuous symptoms (headache vertigo angina pectoris, breathlessness)			5	6
Known hypertension	13	65	33	42
Total	20		79	
Active hypertensive treatment				
Never tried	4		6	
Tried but dropped	3		10	
Maintained but inadequate	3		10	
Maintained	3		7	

tients the other seven all had arrhythmia due to arteriosclerotic heart disease There was only one patient with operated valvular disease (mitral stenosis and insufficiency) Prophyalctic dicumarol treatment had been given to three patients but in one of them had therapeutic anticoagulatory levels been achieved

DISCUSSION

The results show a further decrease of cerebral haemorrhage when recalculated upon the whole population at risk The number of cerebral embolisms was about the same as in 1964

When considering the age distribution, it is seen that a reduction has taken place in the ages below 60 for both haemorrhages and encephalomalacias Above the age of 60 there was an increase indicating that the incidence of stroke has been pushed upwards in age There were scarcely any cerebrovascular lesions at all below 50 years. Within the age group 60–69 there is still a tendency to progression towards the higher age as most of the cases were aged 65–69

One source of error in this investigation may be that the records of the Coroner s Office have not been sought for deaths due to cerebrovascular lesions However most people live some time after a stroke and are then admitted to hospital especially in a city with a well developed ambulance service as in Göteborg It must also be noted that figures from the Coroner s Office were not included in the previous clinical investigation here used for comparison. The minor number of patients who die in their homes and have a death certificate without autopsy from their physician may be supposed to be unchanged from 1949 to 1964

A downward trend for the mortality in stroke was noted even before the era of active antihyper tensive treatment due to improved care anti biotics, etc. This however does not interfere with the results of this investigation

It is a striking fact that still many people with known hypertension either have never been treated or the treatment has been allowed to get com pletely out of order (Fig. 3). The reasons may be lack of initiati e and determination in treatment or aggravating factors such as alcoholism or psychiatric abnormity of the patient One patient forgot his medicine when going on a journey two others stopped the treatment because the med cine stores were not refilled Even when treatment was continued, it must still be considered really inadequate in many patients

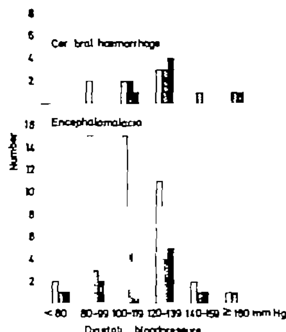


Fig 3 Diastolic BP on admission and antihypertensive treatment in the 1964 series. □=previou BP unknown or normotensive ▨=treatment never tried or dropped. ■=treatment maintained

Table II *Analysis of complicating conditions previous antihypertensive treatment and mean diastolic BP on admission in patients with cerebral haemorrhage and encephalomalacia*

Number of deaths given within parentheses

	Antihypertensive treatment maintained		Antihypertensive treatment dropped or never tried		Previous BP unknown or normal	
	No. of pats.	Diastolic BP	No. of pats.	Diastolic BP	No of pats.	Diastolic BP
Haemorrhage						
No complicating conditions	4 (3)	105	6 (5)	120	5 (5)	105
Non-haemorrhagic complications	2 (2)	115	1 (1)	120	2 (1)	110
Encephalomalacia						
No complicating conditions	13	112	3 (2)	110	43 (3)	90
Haemorrhagic complications	2	107	1	100		
Non-haemorrhagic complications	2	110	12 (1)	105	3	100

Valvular heart disease contributed to nearly half of the cases with embolism Only one operated patient was found in this group Earlier investigations by Wood (9) have shown that 9–14% of patients suffering from rheumatic valvular disease have a history of embolism, 60% of them are cerebral

It is striking that all cases of embolism, regardless of origin occurred in patients without or with inadequate anticoagulant treatment. In an analysis of 754 patients with rheumatic heart disease Szekely (8) found that anticoagulant treatment reduced the incidence of embolic recurrences The results of the present investigation also stress the importance of adequate embolic prophylaxis.

Uraemia was found only in two cases neither of them was fatal There may be many possible explanations of this. A more active treatment of hypertension may be the principal reason Earlier tracing and treatment of pyelonephritis and glomerulonephritis are also certainly of importance The restricted purchase of phenacetin-containing analgesics has undoubtedly reduced the frequency of non-obstructive pyelonephritis

The still continuing trend of decreased incidence of stroke after more than two decades of available active antihypertensive treatment is promising. But it is a sad reality that so many patients are still not satisfactorily treated. It is reasonable to conclude that a far greater benefit is to be gained from an active aggressive attitude in the treatment of hypertension as well as other predisposing factors.

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INSULIN RESPONSE TO INTRAVENOUS GLUCOSE DURING LONG-TERM TREATMENT WITH PROPRANOLOL

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Abstract The early insulin response to i. glucose has been studied in 10 patients with primary hyper tension before and during long-term treatment with large oral dosage of propranolol. N change in the early insulin secretion was found.

In recent years the β-adrenergic blocking agents have become widely used in the treatment of hypertension. These drugs are well tolerated by the patients. No serious adverse metabolic effects have so far been noted but propranolol has been reported to attenuate the acute insulin response to glucose (1 3 4) This might be of interest since a defective early insulin response has been postulated to be characteristic of prediabetes (2)

The aim of the present study was to investigate whether any change in insulin response to glucose occurs during long-term treatment with large doses of propranolol

MATERIAL

The material consisted of 10 men, aged 50 with hyper tension discovered at health examination survey The criteria for hypertension was a diastolic BP of ≥105 mmHg on at least two occasions in recumbent position. BPs were measured in the morning after 10 min rest. The same subjects were reinvestigated during treatment with propranolol (Inderal® ICI England) used alone as therapy

METHODS

The glucose tolerance tests (IVGTT) were performed in the morning after an overnight fast and with the subjects at rest. The IVGTT were performed before and during propranolol treatment.

The glucose stimulation was provided by i. administration of 0.5 g glucose/kg b.wt. as 50% solution injected during 2 / min

Serum insulin levels were measured in blood samples drawn at 0, 4 6 8 and 60 min after the beginning of the glucose injection. Insulin was determined with the Phadebas Insulin Test (Pharmacia Uppsala, Sweden) based upon the radioimmunosorbent technique described by Wide et al. (6). The mean value of the insulin levels in serum at 4 6 and 8 min and the difference between this value and the basal insulin level (Δ4–8 min) were calculated.

Blood samples for determination of glucose in plasma were taken at 10-min intervals during one hour for estimation of glucose tolerance, which was expressed as K-value calculated from the formula $K=$ (1 $2\times100)/T\frac{1}{2}$, where $T\frac{1}{2}$ is the time (min) required for the concentration to be reduced by half its value.

RESULTS

The mean duration of treatment was 11 months (range 5–20) and the average daily propranolol dosage 510 mg (range 240–1000) No other drugs were given. There was no change in body weight between the two tests

Mean values for fasting serum insulin before and during treatment with propranolol as well as the late insulin response to i v glucose expressed as serum insulin at 60 min after the glucose injection are shown in Table I The differences were not significant.

The early insuli response expressed as mean Δ4–6–8 min is also shown in Fig. 1 A wide distribution is seen, but there was no inhibition of the insulin response The correlation between the two values in the same patient was low ($r=$ 0.35) and not significant ($p>0.05$)

There were no significant differences between the mean values of the two investigations with regard to glucose disappearance rates (Table I)

Table I Serum insulin (μU/ml) levels and glucose disappearance rates in 10 men before and during propranolol treatment (mean ± S D)

Insulin level	Before treatment	During treatment
Basal	13 7±6 0	14.₋±5.3
Δ4-6-8 min	71.5±30.5	66.9±32.
60 min	41 9±33 6	49 1±22.0
K-value	1 68±0 78	1 42±0 48

DISCUSSION

The main purpose of antihypertensive treatment is to reduce the incidence of cardiovascular catastrophes Hypertension is only one among many factors which promote the development of cardiovascular disease Increased serum lipids and impaired carbohydrate tolerance are other such factors known from various epidemiological studies It is essential that the treatment designed to influence one of these factors does not cause metabolic alterations which influence another in an unfavorable way The reports by Cerasi et al (1 3) that the initial insulin response to i v glucose is impaired after propranolol are alarming This type of impairment in insulin response has been considered prediabetic (2). Thus the antihypertensive effect of this drug could be counterbalanced by the changed insulin secretion pattern

The proper means to study this effect on insulin secretion should be to compare the insulin response before and during long term oral medication of propranolol in hypertensives However all previous studies with regard to the release of insulin have been performed as short-term tests after parenterally given propranolol What is clinically important with regard to the overall preventive aspect of this effective antihypertensive drug is its influence on carbohydrate and insulin metabolism during long-term oral treatment.

The present study has not shown any alteration in glucose tolerance and insulin response to i v glucose during treatment with massive oral dosage

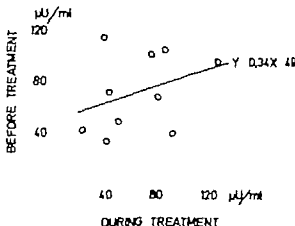


Fig 1 Early insulin response (Δ4-8 min) to i. glucose before and during long-term treatment with propranolol

of propranolol. This is in accordance with a recent report (5) that the insulin secretion is independent of β-adrenergic receptors.

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PREHOSPITAL PHASE OF PATIENTS ADMITTED TO A CORONARY CARE UNIT

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Abstract The interval between onset of chest pain and admission to Stockholm Coronary Care Unit (CCU) has been investigated in 137 patients, 42% of whom were in the CCU within 3 hours (median about 3½) of onset of symptoms. The main part of the interval was patient delay 30% calling for help within 30 min, yet as many waiting for 3 hours or more before doing so Of those seeking help within 30 min 85% were in the CCU within 3 hours of onset of symptoms Almost half the patients sought help by calling the Central Health Information Centre and 84% of patients were sent to hospital without first being seen by a physician. The subsequent diagnosis, i.e acute myocardial infarction or not, did not affect the delay Factors in the past medical history associated with shorter delay were previous CCU care and "visit to physician during last year Delay was also shorter for patients not at home at the time of onset of symptoms and for those who encountered a positive attitude when calling for medical help. For unknown reason there were significantly more patients with anterior infarcts among those with short delay Sex age and self-diagnosis did not affect the delay

Most patients who die of an acute myocardial infarction (AMI) do so outside a hospital (1 3 9) Therefore several groups of workers have analysed the time elapsing between onset of symptoms of an AMI and admission to hospital (2 3 5 6 7 8 11 15 16 19). This prehospital phase or "delay poses a major challenge and raises numerous questions as to possible therapeutic approach as well as feasibility of reducing its length. As both geographic and organizational differences must be taken into account when studying this phase we thought it worth-while to evaluate admission delay in Stockholm in order to find out which factors affect it

Delay is commonly subdivided into 1) patient delay or "decision time) doctor s delay 3) transport delay and 4) hospital delay Amongst others Tjoe and Luria (19) have stated that the major part of the delay can be attributed to the decision time" i.e the interval between onset of symptoms and the patient s decision to seek medical help These subdivisions of total delay no doubt serve their purpose However we feel that the patient, his personality his powers to express himself and his knowledge of his ailment can affect all parts of the delay period Total delay as well as its subsections have therefore been analysed

METHODS

Serafimerlasarettet, with 200 medical beds and 7-bed CCU serves defined part of Greater Stockholm. Organization, admission and diagnostic criteria for the CCU have been given elsewhere (18)

For purposes of this study only consecutive patients with central chest pain as the dominant symptom have been included. Further criteria were capability of giving a coherent history survival after admission until an interview with one of the authors within 24 hours of admission, and onset of symptoms outside the hospital. Patients with longer delay than 48 hours are not admitted to the CCU The material was collected during the autumn of 1971 Patients who following the ECG and routine enzyme tests, received the diagnosis of an AMI will be referred to as AMI patients and those who did not non-AMI patients

In Stockholm patients who feel suddenly and severely ill do not in most cases contact general practitioner but rather the Central Health Information Centre, with an easily remembered telephone number 90 000 which also covers other emergency services like ambulance fire brigade and police This central office is staffed by nurses and sends emergency calls by radio to chauffeur driven doctors. If clear history of central chest pain is given the nurses will immediately send an ambulance to the patient for direct transport t hospital The patients are brought to the hospital serving the district where they are domiciled. A salient feature in this study i also that considerable proportion of our admissions are not first admissions. patients there

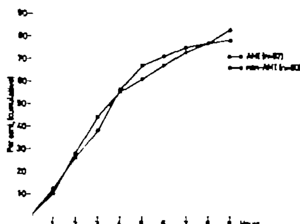


Fig 1 Delay in admission expressed as admissions per hour (cumulative percentage) during first 9 hours from onset of chest pain

fore should know what to do where to go, and they often first phone the CCU to ask for advice.

A point which needs to be answered is what constitutes a "reasonable delay in this community Considering the time taken as regards the effect of nitroglycerine telephone call for medical help ambulance to the patient transport to hospital the time necessary for a preliminary appraisal in the Casualty Department and transport to the CCU a delay of 2 hours is adequate and of 3 hours perhaps reasonable whereas longer delays strongly suggest a major interfering mechanism Patients who had arrived within 3 hours of onset symptoms, i.e. "short delay patients will in the sequel be referred to as SDPs and those with longer delay as LDPs.

MATERIAL

After exclusion of 7 patients with an equivocal diagnosis and 5 who died before an interview could be arranged there remained 137 patients, 57 with a subsequent diagnosis of AMI and 80 in whom this diagnosis could not be proven, i.e. non-AMI patients. On the other hand 64% of the latter had a history of ischaemic heart disease (IHD) The age and sex distribution is shown in Table I. Three of the AMI and one of the non-AMI patients died during hospitalization.

Table I *Age and sex distribution of the 137 patients investigated*

	AMI (n=57)	Non-AMI (n=80)
Age (y)		
Mean	65.5	63.3
Range	50-92	34-81
Sex		
Men	43	52
Women	14	28
Ratio	3 1	1.9

RESULTS

General findings The delay periods of the AMI and non-AMI patients are given in Fig 1 showing that the two groups behaved similarly in this respect. Adding the two groups together the following figures emerge 27% were admitted within 2 hours 42% within 3 hours and well over half the patients (56%) were in the unit within the 4th hour after onset of chest pain

Decision time (patient delay) (Table II). Forty patients (29%) 20 (35%) AMI and 20 (25%) non-AMI or someone in their surroundings acted within 1 hour by demanding medical aid in some form (ambulance doctor or nurse) Not surprisingly this type of rapid action was associated with a high 3-hour (SDP) admission rate of 85% About half of the patients delayed seeking medical help by 1 1 hours or more after onset of symptoms The mechanism of patient delay was investigated in the 71% with a decision time exceeding 30 min. Of these patients 33% thought that their delay was due to a "thought pain would pass mentality 11% thought their symptoms not serious or just due to another bout of their angina, and 7% thought the pain was extracardiac in origin. Only 7% did not want to disturb and 1% felt too paralysed to call for help Vague reasons not referable to any of the above were given by 12%.

Table II *Patient delay (decision time) of the 137 patients before admission to CCU*

	Help called within					
Patients	0–15	16–30′	31–60′	61–120′	121 180′	≥3 hours
AMI	7	13	6	9	5	17
Non-AMI	9	11	9	15	10	26
All (cumulative percentage)	12	29	40	58	69	100

Table III *Attitude to call for help encountered from first contacted medical personnel*

Attitude encountered	Patients No.	%
Type 1	76	55
Type 2	5	4
Type 3	30	22
Type 4	26	19

No differences in these respects were found between AMI and non-AMI patients

The person who called for medical help was in 36 instances (26%) the patient himself It was observed that 31 (39%) of the non-AMI called for help themselves as compared to only 5 (10%) of the AMI patients ($p<0.001$)

Pain relief was resorted to by 67% of the patients 36% took nitroglycerine 1% alcohol 9% salicylates, 7% inappropriate drugs, e.g. digitalis and dicoumarol and 15% combinations of the above SDPs and LDPs and also AMI and non-AMI patients, when compared did not differ from each other in these respects

Medical help was sought by ringing the Central Health Office in 44% of the cases, their own physician in 12% a nurse in 4% whereas 16% went directly to hospital with the help of a relative or friend or occasionally on their own Direct contact with the hospital (usually the CCU) was taken by 24% None of these primary medical contacts were related to any specific pattern as regards delay nor did any method differ from the others as regards relative numbers of SDPs.

Attitude of first medical personnel contacted

Table IV *Interval as estimated by the patients between call for transport and departure for hospital*

Time	No. of pats.	Cumulative percentage
0–15 min	44	32
16–30 min	39	61
31–60 min	17	73
1–2 h	10	80
2–4 h	6	83
≥4 h	4	88
Uncertain (own car or other form of transport)	17	(12)
Total	137	100

Table V *Hospital delay between Casualty Department and CCU*

Delay (min)	Patients AMI	Non-AMI	All (cumulative percentage)
0–15	30	31	45
16–30	11	23	69
31–60	6	15	85
61–120	7	6	94
≥120	2	4	99
Uncertain	1	1	100
Total	57	80	100

The advice given by the medical personnel first contacted could be classified into 1) advice or even help to obtain an ambulance immediately for transport to hospital 2) similar advice but suggesting taxi or car 3) delaying, e.g. arranging for a physician to come to the patient first or asking the patient to wait to see whether pain improves and 4) unknown. As similar attitudes were encountered by both the AMI and non-AMI patients, they are accounted for together (Table III) Not surprisingly the most salutary type of attitude encountered (type 1) does affect delay as 67% of the 57 SDPs met this attitude as compared to 48% of LDPs ($p<0.05$) Accordingly a delaying attitude (type 3) was met by 31% of LDPs as compared to 14% of SDPs ($p<0.05$) A doctor's delay was present in 16%

Interval between call for medical aid and departure for hospital Table IV shows that 61% of the patients both AMI and non-AMI thought that they had obtained transport within *1* hour of request. Ambulance transport was employed by 83 (61%) and taxi or private car by 40 (29%) of the patients. Exact time estimates for the transport to hospital were not available

Delay in hospital between Casualty Department and CCU The time elapsing between arrival in hospital and in the CCU is presented in Table V Altogether 45% of the patients had a shorter hospital delay than 15 min. Reasons for prolonged stay in the Casualty Department were investigated Age was found not to matter but final diagnosis did Of the AMI patients 60% were in the CCU within 15 min of arrival in the hospital against 36% of the non-AMI patients ($p<0.01$).

Previous medical history in relation to total delay Angina pectoris had occurred in 83 (61%)

of the patients equally represented in SDPs (60%) and LDPs (61%) Similarly a previous AMI was of no significance 37% of SDPs and 31% of LDPs gave a history of previous infarctions Hypertension was reported by 35% of SDPs and 30% of LDPs and the corresponding figures for a history of congestive heart failure were 32% and 36% respectively Also diabetes mellitus occurred about equally in the two groups—5 and 6% respectively

The incidence of previous CCU care in the two groups was 37% of SDPs and 25% of LDPs (N S) However it was found that if a 2-hour interval is chosen for definition of short delay then previous CCU care does affect delay Of 41 patients with a history of CCU care 20 (49%) had arrived in the CCU within 2 hours against 23 (24%) of the 96 patients with no previous CCU care ($p<0.01$)

Delay was also related to visits to a physician during the last year A majority of patients had in fact been to see a physician during the last year at least once 91% of SDPs and 78% of LDPs ($p<0.05$) Regular visits were reported by 39% of SDPs and 30% of LDPs (N S.) Medical visits because of IHD per se were reported by 58% of SDPs and 48% of LDPs (N S)

Personal factors Psychological factors and those related to education will be published elsewhere (10) Age sex and marital status did not affect delay times significantly Similarly heredity factors seemed non-significant—37% of SDPs had a heredity of IHD against 39% of the LDPs Nor did awareness of myocardial infarction as measured by known contacts with a person with this disease during the last year (fellow patients in the CCU excepted) differ in the two groups Of the SDPs 21% were aware of contacts with a person with AMI and 13% of the LDPs

The relationship between delay and how patients perceived their own diagnosis was also investigated. A cardiac self-diagnosis was made by 65% of the patients No difference in this respect was found between SDPs and LDPs (68% and 63%, respectively) Nor did a self-diagnosis of AMI affect delay—47% of SDPs thought they had an AMI as compared to 31% of LDPs. This difference falls just short of statistical significance It should be remembered that these figures were obtained in a CCU—surroundings which well might affect a patient s diagnostic considerations

External factors Physical activity at onset does not appear to influence delay 39% of SDPs were asleep or resting against 51% of LDPs (N S) Being at home was more common (81%) among the LDPs than among the SDPs (65%) ($p<0.05$) This difference was essentially due to the non-AMI LDPs nearly all of whom (91%) had been at home Those with a subsequent diagnosis of an AMI did not differ from the SDPs in this respect 69% having been at home at the time of onset of symptoms

Delay times were not related to weekdays or holidays Among the SDPs 60% reported their onset of symptoms to have begun between 8 00 a.m and 8 00 p m. but this does not differ from 48% for the LDPs The presence of a spouse or other person at the time of onset of symptoms did not seem to affect delay times Thirty-eight per cent of SDPs and 34% of LDPs were alone at the time of onset of symptoms (N S)

Relationship between clinical features and delay Anxiety did not seem to affect delay In fact it was more often reported by the LDPs (49% against 35% N S) Nor did the type of pain (pain burning sensation oppression etc) seem to matter and the same was found concerning radiation of pain—67% of SDPs and 75% of LDPs mentioning this feature Associated autonomic symptoms also failed to shorten delay—75% of SDPs and 81% of LDPs reported symptoms including cold sweat, frozenness nausea or vomiting Arrhythmic sensations occurred in 43% of LDPs and 33% of SDPs (N S) Dyspnoea would appear to be an alarming symptom but was reported by 42% of SDPs and 45% of LDPs Thus LDPs seemed to be subject to a slightly more marked symptomatology rather than the other way round

More objective parameters of the acute condition also failed to differentiate between SDPs and LDPs among which admission systolic BP heart rate and findings of congestive heart failure. That the ultimate diagnosis did not affect delay has been mentioned above Furthermore infarction size (AMI patients only) as estimated by SGOT maximum level was similar in SDPs and LDPs Anterior infarction occurred in 45% (10/22) of SDPs with AMI as compared to 17% (6/35) of LDPs ($p<0.05$)

DISCUSSION

To summarize it seems that about half the patients were in the CCU within 3 / hours of

onset of chest pain. These findings correspond to those in several other communities (7 13 15 16), whereas longer delays have also been reported (1 3 8 11). When comparing this study with similar foreign studies, it should be remembered that in the Stockholm area patients with central chest pain are usually sent direct to hospital and doctors delay is often avoided Thus Armstrong et al (1) point to a considerably shorter delay for those who had not been referred via a general practitioner as compared to those who were.

In this evaluation the onset of symptoms has been equated with onset of myocardial necrosis. However se eral nvestigations have shown a marked discrepancy between autopsy f ndings and duration of the terminal attack (12, 14 20) An improved understanding of the earliest phases of an AMI which perhaps in part are characterized by prodromal symptoms" may in the future open the way to a better definition of the true total delay in deal ng with this disease thereby allowing for quicker intervention

*The dominant portion of the delay is the "deci*sion time (1 8 11 19) Like Sandler and Pistevos (13) we found that about one third of the patients call for medical assistance within 30 min. Our results are also similar to those of Armstrong et al (1) and of Gilchrist (4) whereas longer decision times are given by others (8 11) Moss et l (11) gi e age as one factor affecting the decision time a f nding not demonstrable in the present study

Hospital delay figures have previously been reported by Tjoe and Luria (19) It is clear from the present study that, in spite of instructions to the Casualty Department staff 30% of patients remained there for 30 min or more. On the other hand means of resuscitation do exist in the Casualty Department, reducing the importance of this time loss during which treatment is commonly started It was found that a final diagnosis of AMI was related to a more rapid transfer to the CCU Clearly special efforts are made by the staff in cases in which an AMI seems likely

Generally the present findings appear disappointing. The factors found to affect total delay include not at home previous CCU care anterior location of the infarction and visit to physician during the last year' W th the exception of the latter these findings are of limited practical use It is perhaps more interesting that patients with a history of diabetes hypertension, angina and myocardial infarction are not overrepresented among those with shorter delay These patients are easily available for education It must be borne in mind, though that t has not been shown that education shortens delay Improved knowledge may strengthen a protective neurotic process of denial" and thereby produce the opposite of what is intended Denial as well as other factors involved in the delay process have recently been discussed by Yu (21) who also mentions the possible salutary role of family members and friends in reducing delay The significant role of a second person, especially strangers in initiating the decision to seek help has also been pointed out by Hackett and Cassem (7)

Of more practical use is the finding though not surprising, that a positive attitude on the part of the first medical personnel contacted does seem to affect total delay Failure to arrange for immediate ambulance transport clearly lengthens the total delay It must be concluded that in this type of patients with central chest pain the response of the medical personnel first contacted should be to organize rapid admission to a CCU

Other delay lengthening factors include week ends and daytime (11) time of day (1 ... 5) preceding crescendo angina (15) and a history of cardiac disease or misinterpretation of symptoms as being a recurrence of angina (6) There was no indication i the present study that any of these factors had a significant influence Nor did Tjoe and Luria (19) consider that a history of AMI affected delay They also found that a self diagnosis of AMI does not shorten delay which s in accordance with the present study This finding again raises doubts as to the potential efficacy of educational measures which would be expected to provide information enabling patients to diagnose their condition. Yet a self-diagnosis of AMI apparently does not shorten the delay significantly perhaps again suggesting denial mechanism. Alternati ely although capable of diagnosing a possible AMI patients are genuinely naware of the severe threat to their lives during the early phases of this condition

A factor found to be unrelated to delay was the severity of the acute attack as measured by the Peel score whereas subjective assessment is related to delay (7) Recently Barber et al. ( ) found

a relationship between severity of attack and patient delay In the present study severity was not measured per se but different symptoms and clinical findings associated with an AMI played no significant role in influencing delay A curious finding was an overrepresentation of anterior infarctions amongst SDPs. In this context it is of interest to note that differing symptomatology has been reported when comparing anterior infarctions with those of other localization (17)

As in other similar studies the present investigation was based on information gathered from the survivors of the most crucial early period and some caution must be exercised when transferring the results to those who die early never reaching a hospital This latter group is of interest in evaluating early phases of an acute manifestation of IHD Relevant figures in the Stockholm area are given in Wikland s monograph (20) where a very high mortality rate within the first 15 min (61% for males and 48% for females) is reported These patients may be considered beyond medical reach with present means except in special circumstances where there may be resuscitation facilities i e. industrial plants sport stadiums etc A poor yield remains likely as some 70% of cases report onset of symptoms in their homes as was found in this study as well as by others (9)

A feasible aim may perhaps be to have the majority of patients in this community in a CCU within 2 hours of onset of symptoms. This is not impossible as is shown by the 27% of our patients who do arrive within this time With this goal in mind, and with reference to the figures given by Wikland (70) and Kuller et al (9) it might be possible to bring about 25% of those who at present die medically unattended outside hospitals, to proper care This figure however presumes that these patients have a recognizable symptomatology

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LATE RISE IN SGPT AFTER ACUTE MYOCARDIAL INFARCTION

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Abstract The present study draws attention to late rise in SGPT following acute myocardial infarction (AMI) beginning about 4 days after the SGOT max. This observation is illustrated by an investigation of 7 patients, selected because of this finding, and 7 controls, also with an AMI but without this enzyme pattern. Peak SGOT and LDH enzyme levels during the acute phase were similar in the two groups. The late SGPT rise was associated with high serum alkaline phosphatases 2 weeks after the AMI but not 5–9 months later Two weeks after onset of symptoms BSP excretion was commonly impaired but failed to separat the two groups. I contrast to the controls, BSP excretion was significantly improved at reinvestigation 5–9 months after the AMI in the group with the SGPT rise.

Impaired hepatic function in patients with chronic congestive heart failure (CHF) is well recognized (4 8 11 13 16 19 22 4 28 29) but little attention has been paid to this in AMI patients (1 18) Concomitant with a diminished excretory capacity for bromsulphalein (BSP) there is often also other laboratory evidence of hepatic impairment in chronic CHF (3 11 13 14 16 22 24 28 29 30) Changes in liver perfusion, anoxia raised venous pressure as well as malnutrition have been advanced as possible causal mechanisms (11 19 23 28).

In uncomplicated AMI rises in serum alanine transaminase (SGPT) roughly follow the rises in serum asparaginic acid transaminase (SGOT) but do not increase above the upper normal level unless the infarction is extensive. Sources other than the heart should therefore be suspected cases with unexpected SGPT rises in the early phase (4 30) Excessively high levels—more often seen when severe right-sided heart failure shock or pulmonary embolism complicate myocardial infarction—are thought to indicate liver cell injury (3 17) and can at times jeopardise the serum enzyme diagnosis of AMI (4 5)

The present investigation of patients with AMI was initiated by repeated observations of a late rise in SGPT beginning about a week after onset of symptoms and exceeding simultaneous SGOT values. This rise was usually not accompanied by a rise in SGOT and was therefore assumed not to reflect reinfarction, nor was there any clinical evidence of this being the case In an attempt to clarify this enzyme rise a further evaluation was made of hepatic function in 14 patients with AMI 7 with and 7 without this enzyme rise

METHODS

The patients were treated in the Coronary Care Unit (CCU), Serafimerlasarettet. The criteria for admission and diagnosis, policy of treatment etc. have been given elsewhere (25). SGOT SGPT LDH and LDH (the heat stable portion of LDH) were analysed routinely on admission and every 12 hours while the patients were in the CCU and twice week for the remainder of the hospital stay and were repeated at the time of the liver function studies. Analyses were made with Reaction Rate Analyzer (LKB 8600) connected to an evaluation unit (Optilab, Bo Philip Instrumentation, Stockholm) (6, 26). Normal upper levels for SGOT SGPT and serum alkaline phosphatase (AP) were <40 mU/ml and for LDH <350 mU/ml. A standard BSP excretion test (5 mg/kg b.wt.) was performed about two weeks after onset of the AMI and again 5–9 months later A retention of 5% or more after 45 min was considered pathological. At the time of reinvestigation the patients were also questioned regarding their alcohol consumption

The definition of this late secondary rise in SGPT after AMI for the present study was a rise in the enzyme exceeding simultaneous SGOT levels beginning 4–5 days after SGOT maximum This implies that the enzyme rise starts about one week after onset of symptoms and that the SGPT need not rise to pathological levels

Table I *Clinical findings in the study group and the controls*

Ap=angina pectoris, Db=diabetes, Ht=hypertension, MI=myocardial infarction

Case no.	Age (y)	Sex	History of CHF	Ap	Db	Ht	MI	Infarction site	Heart size (ml/m /BSA)
Study group									
1	64	♂	–	–	–	–	–	Inferolateral	540
2	71	♂	–	–	–	–	–	Inferolateral	710
3	55	♂	–	+	–	–	–	Inferolateral	370
4	66	♂	–	+	–	–	–	Inferior	430
5	64	♂	+	+	–	–	+	Inferior	370
6	73	♂	–	+	–	–	+	Inferior	480
7	68	♂	–	–	–	–	–	Inferior	380
Control group									
1	81	♀	–	+	–	+	–	Anterolateral	310
2	60	♂	–	–	+	–	–	Inferior	760
3	56	♂	–	+	–	–	–	Anterolateral	330
4	71	♀	–	+	–	–	–	Inferolateral	380
5	61	♀	–	+	–	+	–	Inferior	510
6	62	♀	–	–	–	–	–	Inferior	350
7	63	♀	–	–	+	–	–	Anterior	420

PATIENTS

The present study presents the findings in 14 non-consecutive patient with transmural AMI 7 with a secondary SGPT rise (study group) and 7 without this enzyme pattern (control) Each time a patient with secondary SGPT rise w s fou d, a control—treated in the unit at the same time but without evidence of similar enzyme pattern—was selected. All patients were treated in the nit from May to Sept. 1972.

The clinical characteristics of the patients are given i Table I The groups were similar as regards mean age, history of heart failure angina pectoris, diabetes and hypertension Two patients in the study group but none of the controls had sustained a previous myocardial infarction. N history of liver disease or alcohol abuse was given.

During the early phase of the AMI 5 patients in the tudy group but none of the controls had ventricular dysrhythmias, wherefore the former received more anti-

Table II *Enzyme values in the study group and in the controls*

Case no	SGOT max. (mU/ml)	SGPT at SGOT max. (mU/ml)	LDH max (mU/ml)	LDH_1 at LDH max. (%)	AP max. (mU/ml)	SGPT max. (secondary rise) (mU/ml)	SGOT at SGPT max. (mU/ml)
Study group							
1	195	33	1 030	90	68	81	55
2	205	40	1 000	84	35	83	49
3	179	47	1 300	87	54	123	100
4	253	51	1 340	87	28	87	59
5	112	31	625	78	44	80	42
6	65	21	410	81	36	35	26
7	62	20	420	76	26	33	21
Control group							
1	138	39	730	78	26	–	
2	350	42	1 500	82	13	–	–
3	89	20	485	68	22	–	
4	143	21	720	85	38	–	
5	87	17	430	86	27	–	
6	141	24	750	85	30	–	
7	86	21	700	77	18	–	
p*	N.S	N.S.	N S	N S	<0.025		

* Study group—control group.

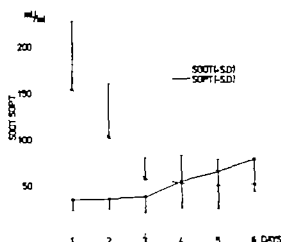


Fig. 1. Enzyme pattern of SGOT and SGPT in 7 patients with secondary rise in SGPT. Day 1 corresponding to recorded SGOT maximum.

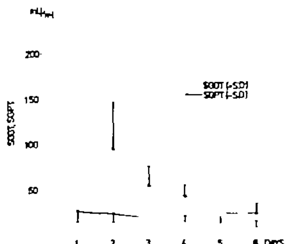


Fig. 2. Enzyme pattern of SGOT and SGPT in 7 control patients. Day 1 corresponding to recorded SGOT maximum.

arrhythmic drugs, but the groups were similar with respect to other treatment including digitalis, diuretics, analgetics, sedatives and anticoagulants. CHF was noted in 6 patients of the study group and in 4 of the controls. One patient in the control group was hypotensive and had also the highest AP value and the most markedly impaired BSP excretion. At discharge 3 patients in the study group and one of the controls were on digitalis and at the time of the reinvestigation the number had risen to 5 and 2, respectively. No patient had had reinfarction in the meantime.

All patients in the study group and 4 of the controls had ECG signs suggesting involvement of the inferior wall of the left ventricle. The mean heart size at discharge was 469 ml/m² BSA in the study group and 437 ml/m² BSA in the controls (N.S.).

RESULTS

Laboratory findings. The serum enzyme levels are given in Table II. The mean maximum SGOT

Table III. *Liver function tests and enzyme values (mU/ml) in the study group and in the controls*

Case no.	Enzymes at first BSP test SGPT	SGOT	AP	First BSP retention after 45 min (%)	Enzymes at second BSP test SGPT	SGOT	AP	Second BSP retention after 45 min (%)
Study group								
1	22	20	46	18	13	15	20	7
2	20	37	26	16	19	14	12	8
3	88	48	35	6	12	16	13	4
4	53	21	28	13	18	21	19	7
5	40	20	31	7	17	20	1	7
6	15	13	30	16	13	21	37	12
7	34	18	22	10	15	17	14	8
Control group								
1	17	34	15	25	10	18	34	12
2	12	15	12	7	11	20	12	6
3	26	29	17	6	16	18	22	5
4	12	14	38	8	13	19	22	8
5	13	16	27	8	10	14	16	8
6	10	13	31	3	10	15	22	3
7	13	16	17	3	11	16	22	
p^a	<0.025	N.S.	<0.05	N.S.	<0.01	N.S.	N.S.	N.S.

ᵃ Study group—control group.

In the study group was 153 mU/ml and in the controls 148 mU/ml The mean SGPT in the study group at the time of maximum SGOT was insignificantly higher 35 mU/ml as compared to 26 mU/ml in the controls The secondary SGPT rise exceeded the normal level in 5 of the study group patients being within the normal range in 2. The enzyme patterns are shown in Figs 1 and 2

The SGOT SGPT and AP values at the time of the first BSP test performed 10–17 days after the AMI are given in Table III The groups still differ in SGPT Also AP levels were significantly higher in the study group ($p<0.05$) but the difference in BSP retention was not (means 12.3% and 8.6% respectively) All patients except two of the controls had abnormal BSP retention

At the time of the second BSP test 5–9 months after the AMI the SGPT values were within the normal range but still significantly higher ($p<0.01$) in the study group but no differences were found in AP SGOT or the BSP test. If liver function at the time of the first BSP test is compared to that at the time of the second no differences were found within the control group In contrast AP levels decreased significantly ($p<0.05$) and BSP excretion showed a significant improvement ($p<0.02$) in the study group (Table III)

DISCUSSION

Using routine enzyme tests we identified a group of patients with a late rise in SGPT beginning towards the end of the first week after an AMI in contrast to the commonly observed early minor rise that roughly parallels the SGOT rise To find out whether this late SGPT rise was associated with impaired hepatic function the latter was evaluated with standard liver tests including BSP loadings and AP determinations (3 19)

Hepatic function in AMI has been investigated previously Aber et al (1) performed a modified BSP test in the earliest stages of an AMI and found impaired liver function which was related to the clinical condition of the patients They supported the finding of Van der Helm et al (27) that the rise in LDH 5 isoenzyme activity originated from the liver since that enzyme pattern was associated with poor BSP excretion. However Albertini et al (2) proposed that an abnormal BSP test does not necessarily indicate liver cell damage but merely reflects circulatory failure

The relationship between hepatic blood flow and abnormal liver function in chronic CHF remains to be delineated Myers and Hickam (20) found that there was a decrease in hepatic blood flow in chronic CHF roughly proportional to the decrease in cardiac output Somewhat at variance with this Losowsky et al (19) found rather normal hepatic blood flow in such patients even in the presence of severely impaired BSP excretory capacity The findings vary and there are reports (11) of a parallel decrease in BSP excretion and severity of heart failure

A positive correlation between right heart pressures and the BSP test has been noted (11 19 28). In patients with pulmonary insufficiency however no such simple relationship between right-sided pressures and liver damage seems to exist (21)

Collins et al (9) have pointed to an association between a raised central venous pressure, dysrhythmias and inferior infarction This, perhaps has a bearing on the present study as all patients with ventricular dysrhythmias were in the study group all of whom also had inferior infarctions. More antiarrhythmic agents were therefore used in the study group a factor which cannot be excluded as a cause of liver damage However different drugs were employed and they were given at varying stages of the illness

In the study group the BSP values were significantly lower in the second than in the first test. The improvement of AP and BSP excretory capacity suggests improvement of heaptic function with time in these patients Ewen and Griffiths (12) however have proposed that changes in some enzymes e.g. AP could be due to necrotic as well as reparative processes Felder et al (13) suggested that AP was one of the more sensitive tests in CHF but in contrast to the present findings White et al (29) state that AP levels are not corrected with time in patients with CHF Furthermore Betro (7) described increases in AP in patients with AMI but without clinical evidence of heart failure and a hepatic source was assumed

The study group did not differ from the controls as regards clinical signs of circulatory failure or history of CHF Furthermore no differences were noted as regards venous pressure and infarction size as estimated by enzyme maximum. The latter may however be an inadequate measure as regards the relationship between myocardial damage and circulatory impairment Infarction of papillary

muscles as well as right ventricular infarction may be of considerable circulatory significance. It is noteworthy that all patients with a rise in SGPT had inferior infarctions, and these infarctions are particularly prone to right ventricular extension (10).

The present study thus suggests that late rises in SGPT following an AMI are mainly characterized by associated elevated AP levels, whereas the significance of hepatic function remains to be determined.

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ELECTROCARDIOGRAPHIC ARTERIOGRAPHIC AND HEMODYNAMIC CORRELATION STUDIES IN CORONARY HEART PATIENTS WITH ANEURYSM OR AKINESIS OF LEFT VENTRICLE

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Abstract. The results from left ventricular cineangiography have been compared with ECG coronary arteriographic and hemodynamic findings in 59 selected patients with coronary heart disease All patients showed abnormal left ventricular contraction patterns; ventricular aneurysm was present in 22 patients, and ventricular akinesis in 37 All patients with aneurysm had previous history of myocardial infarction and there was close correlation between the ECG pattern and the angiographic findings, while unspecific ECGs were commonly seen in patients with akinesis. All patients had severe coronary artery stenosis. N aneurysm was seen without occlusion of the major local artery while the anatomical location of the arterial stenoses showed less consistent relationship to the angiocardiographic findings in patients with akinesis. Left ventricular end-diastolic pressures were analysed before and after left ventricular angiography and found to be elevated in most patients. No differences were found between patients with aneurysm compared with those with akinesis.

Cineangiography of the left ventricle together with selective cinecoronary arteriography provides valuable information about the functional state of the left ventricular wall and the anatomy of the coronary arteries To-day these two methods have been accepted as useful tools in the evaluation of patients with coronary artery disease

In certain patients with coronary artery disease one may differentiate between two separate ventriculographic patterns: ventricular aneurysm meaning a localized protrusion or angulation of a portion of the left ventricle visible beyond the surface (4) and ventricular akinesis meaning a local absence of ventricular contractions but without abnormal bulges or changes in contour (7) An ordinary cardiac X-ray may occasionally be able to distinguish bulges or changes in contour characteristic of aneurysm but fails to identify an akinetic area which is noticeable only during active systole

Attempts to define and differentiate these two separate ventriculographic patterns have been few In particular there are few studies comparing ECG arteriographic and hemodynamic correlation data in patients with ventricular aneurysm and in patients with ventricular akinesis

MATERIAL AND METHODS

The study is based on the findings in 59 selected patients referred to our Medical Department for diagnostic evaluation of their coronary vessels and left ventricular function with the purpose of cardiac surgery The patients were with few exceptions middle-aged men. A total of 43 patients had a proven history of myocardial infarction, while angina pectoris without known infarction was present in the remaining 16. None of them had overt cardiac failure

Selection of the patients was based on the findings of left ventricular angiograms, which were taken in the right and anterior oblique projection. Only patients with anatomical or functional abnormalities accepted as diagnostic of left ventricular aneurysm or akinesis were included.

Based on the following criteria th patients were divided into four groups *Aneurysm of anterior wall* (16 patients). Clear-cut saccular protrusion of the anterior wall, in many cases also including the apical region Normal contour and contraction of the inferior wall and the constrictor plane (outflow tract to inferior wall). *Aneurysm of inferior wall* (6 patients) Clear cut saccular protrusion of the inferior wall or the dia-

phragmatic region immediately adjacent to the mitral ring. Good contractions in the anterior wall and apical area. *Akinesis of anterior wall* (13 patients) Absence of contractions at the anterior wall and usually also involving the apical region. Good contractions in the diaphragmatic wall. *Akinesis of inferior wall* (24 patients) Absence of contractions in the diaphragmatic region immediately adjacent to the mitral ring and generally extending to the posterior papillary muscle Good contractions in the anterior wall and apical area

Selective cinecoronary angiography was carried out according to the technique described by Judkins (8) The coronary arteriograms were performed in left anterior and right anterior oblique and additional projections when needed The main coronary arteries: the anterior descending branch and the circumflex branch of the left coronary artery and the right coronary artery and their tertiary branches were identified and evaluated separately In regard to the left coronary artery none of the patients examined had a localized severe stenosis of the main stem The structural changes observed were graded according to the classification presented by Malmborg et al. (12) giving each of the three main coronary arteries a score from 0 (no atherosclerotic changes visible) to 3 (total occlusion of the vessel) The three scores were added together to give a total score which represented the overall obstructive disease for these three arteries of the heart.

The contrast material used was Isopaque® Coronar (metrizoate meglumine/Na/Ca (58/9/1) 370 mg/ml) For left ventricular angiography the single dose was 45 ml injected through a Formocath catheter furnished with small end hole and two 1.5 cm long side splits near to the tip The piston pressure of the high pressure syringe was set at 6 kg/cm² For the selective coronary angiographies preshaped catheters (Ducor Cordis Corporation) were used and hand injections of 5–9 ml contrast medium were given repeatedly Cinerecording was performed with a 35 mm Arriflex camera in combination with image intensifier running 75 frames/sec. For preliminary studies a video taperecorder (OD-x40 Old Delft Holland) was used.

Extrasystoles were frequently seen during injection of contrast medium in the left ventricle The assessment of left ventricular contractions was made during stable rhythm.

Left ventricular diastolic pressure was measured before and after 2 min following left ventricular angiography Pressures were recorded using Elema Schönander recording equipment (Transducer EMT 35 and Mingograf 81). The baseline was at the mid-chest level. Left ventricular diastolic pressures were divided into early mid and late diastolic pressures. The late end-diastolic pressure was taken as occurring at the point where the left ventricular systolic wave interrupted the atrial transmitted "a" wave—i e 0 052 sec after the beginning of the Q wave (3)

A 12-lead ECG at rest was taken Based on the ECG findings 18 patients had a normal QRS complex, while 41 had a pathological QRS complex indicating previous transmural myocardial infarction. The infarction was localized as anterior inferior or both using the standard criteria. A more detailed ECG localization was not attempted as the cases in each group were too few

Table I *Clinical diagnosis in 59 patients with coronary heart disease and akinesis or aneurysm of left ventricle*

Left ventricular angiographic findings	Clinical diagnosis: Angina pectoris	Clinical diagnosis: Status post-myocardial infarction
Anterior wall akinesis	6	7
Inferior wall akinesis	10	14
Anterior wall aneurysm		16
Inferior wall aneurysm		6
Total	16	43

RESULTS

Clinical manifestation. Information concerning the previous clinical manifestation of coronary artery disease and the state of the left ventricular function obtained from the ventriculograms is summarized in Table I

Of a total of 37 patients with akinesis 16 (44 %) were without previous known infarction, while the remaining 21 patients in this group, as well as all 22 patients with aneurysm had also a documented previous history of myocardial infarction

ECG findings A normal or borderline ECG at rest was found in 8 of 13 patients with anterior wall akinesis and in 10 of 24 with inferior wall akinesis Thus 18 (49 %) of 37 patients with akinesis had a normal QRS complex The remaining patients in this group had an abnormal QRS complex and the ECG pattern of an old myocardial infarction correlated well with the anatomical location of the akinesis All patients with aneurysm had an abnormal QRS complex In these patients a close correlation was noted when the location of the aneurysm was compared with the location of earlier infarction demonstrated in the ECG

Distribution of coronary arterial disease This series of patients has also been reviewed concerning the presence of single double or triple vessel disease With a few exceptions all patients had either double or more commonly triple vessel involvement as shown in Table II In this respect there seemed to be no difference between

Table II *Relation between left ventricular angiographic findings and severity of coronary artery disease*

Left ventricular angiographic findings	Severity of coronary artery disease			
	Open	Single vessel	Double vessel	Triple vessel
Anterior wall akinesis (*n* = 13)	1		6	6
Inferior wall akinesis (*n* = 24)			6	18
Anterior wall aneurysm (*n* = 16)			4	10
Inferior wall aneurysm (*n* = 6)			1	5
Total	1	2	17	39

akinesis and aneurysm patients. Using the scale of coronary artery stenosis described the relationship between the cineventriculographic findings and the severity of coronary artery changes is shown in Fig. 1 (the small group of patients with inferior wall aneurysm is excluded). The highest percentage of coronary arteries free of atherosclerosis was found in patients with anterior wall akinesis. In this group subtotal or total obstruction of at least one coronary artery was found in slightly more than one half of the patients. Subtotal or total obstructions were more common in patients with inferior wall akinesis or anterior wall aneurysm and were found in approximately 70% of the arteries in these two groups. Both in extent and location the coronary artery lesions bore some relation to the angiographic localization, although the correlation was not a rigid one. This is shown in Table III. The left anterior descending branch was occluded in all but one patient with anterior wall aneurysm. The left anterior descending branch was also the one most commonly involved in patients with anterior wall akinesis but was occluded in only 5 of 13 patients. The posterior descending branch of the right coronary artery or posterior medial branches from the circumflex artery were the most severely affected in 24 patients with inferior wall akinesis and were occluded in 15. The circumflex artery was about equally involved in the four categories of patients. The intergroup differences in total arterial score were not statistically significant.

Hemodynamic findings. In 44 of the 59 patients pre- and postangiographic left ventricular end-diastolic pressures were available. The preangiographic pressures were normal in 8 patients and elevated above upper normal range (10–12 mmHg) averaging 17 mmHg, in 36. There was no significant difference between mean left ventricular end-diastolic pressures in the three main groups (anterior and inferior wall akinesis, anterior wall aneurysm). The postangiographic left ventricular end-diastolic pressures were with a few exceptions higher than those recorded before angiography and rose to an average of 25 mmHg. The pressure rise was of approximately the same magnitude in the three main groups.

DISCUSSION

The relation between coronary artery disease and degree and location of myocardial damage is complicated, as is natural due to the complexity

Table III. *Coronary artery changes in 59 patients with akinesis or aneurysm of left ventricle*

LAD = anterior descending branch left coronary artery. CIRC = circumflex branch left coronary artery. POST = posterior right coronary artery.
Classification of coronary artery disease: grade 0 to 3

Left ventricular angiographic findings	Coronary arterial scores (mean with range)				
	LAD	CIRC	POST	LAD+CIRC	LAD+CIRC+POST
Anterior wall akinesis (*n* = 13)	1.9 (0–3)	1 (0–2)	1.5 (0–3)	3.1 (0–3)	4.7 (0–3)
Inferior wall akinesis (*n* = 24)	1.6 (0–3)	2.0 (0–3)	2.5 (0–3)	3.6 (0–3)	6.1 (0–3)
Anterior wall aneurysm (*n* = 16)	2.9 (2–3)	1.4 (0–3)	1.4 (0–3)	4.2 (0–3)	5.7 (0–3)
Inferior wall aneurysm (*n* = 6)	2.3 (1–3)	1.5 (0–3)	2.1 (1–3)	3.8 (0–3)	5.9 (0–3)

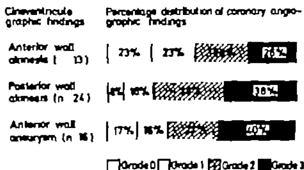


Fig 1 Severity of coronary artery disease Analysis of 53 patients with akinesis or aneurysm of left ventricle. The right coronary artery left anterior descending branch and left circumflex branch are considered. Structural changes graded according to the classification by Malmborg et al. (12)

of collaterals and anastomoses In the present study left ventricular aneurysms were always associated with gross coronary artery disease involving either stenosis or obstructions of the coronary artery supplying the area of the left ventricle where the aneurysm was found The striking predominance of left anterior descending coronary artery involvement with associated anterior or apical aneurysm is in accordance with other studies (13) Most patients with akinesis also displayed marked and diffuse coronary involvement but, contrary to the aneurysm the akinesis did not occur as predicted by the angiographic ation of the coronary arterial disease.

Animal experiments have shown that the extent and severity of the myocardial lesion depend directly on the degree and localization of the coronary occlusion (11) In man this seems to be true in aneurysm but is a less consistent finding in coronary patients with akinesis Although some authors claim that in akinesis there is a very good correspondence between the site of vessel disease and zone of abnormal contractility (5) this was not true either in our series or in a similar study published by Björk et al (2)

All patients with aneurysm showed ECG evidence of old myocardial infarction Interestingly however no evidence of ECG abnormalities at rest were found in approximately 50% of patients with akinesis Several explanations may be offered in this connection It is possible that the resting ECG often fails to reveal a small infarct manifested in ECG as a left ventricular akinesis as suggested by Lichtlen (10) Another possibility is that the muscle zone which fails to contract normally has not yet infarcted due to prolonged ischemia. Repeated sublethal ischemia may destroy sensitive structures essential to excitation-contraction coupling and spare the contractile protein matrix itself (7) The possibility that the akinesis occurs as a result of injection of contrast medium into the left ventricle cannot be excluded, but is improbable as this effect can only be observed 6–10 sec after injection (1)

The observation that development of an aneurysm is critical from a hemodynamic point of view is well documented (7 9) According to Klein et al (9) the involvement of more than 20% of the ventricular surface by non-contractile tissue will result in an increase in left ventricular end-diastolic pressure while the size of an akinetic area assumed to be critical from a hemodynamic point of view is uncertain

In our patients the left ventricular end-diastolic pressure was abnormal in the great majority of patients in whom this parameter was measured and the difference between left ventricular end-diastolic pressures in patients with akinesis compared with those with aneurysm was small and insignificant. The rise in end-diastolic pressure after intracardiac injection of radiographic contrast material represents a stress test of left ventricular performance (6) The postangiographic pressures were of about the same magnitude in the two groups of patients. Taken together our observations seem to indicate that akinetic areas of the left ventricle have a similar effect on left ventricular end-diastolic pressure as aneurysm The statement that left ventricular akinesis in itself may cause heart failure however remains to be proved and needs more extensive investigations

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SERUM LEVELS AND RENAL EXCRETION OF DIGOXIN IN THE ELDERLY

A Comparison Between Three Different Preparations

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Abstract. Bioavailability of digoxin from three different preparations (S, W and L) has been studied by radioimmunoassay of serum and urinary digoxin in a cross-over study. In 25 elderly patients preparations W and L caused significantly higher serum digoxin levels than S, but did not differ from each other in the rapidity of the change or the levels reached. Serum and urinary digoxin of 11 patients were measured on two consecutive days, 10–14 days after changing the preparation. The daily urinary excretion of digoxin is a good criterion of absorption and seems to be in a certain balance with digoxin in the serum. Creatinine and digoxin clearances were positively correlated. In spite of the relatively low creatinine clearances of these elderly patients the better absorption of preparations W and L was greatly compensated by increased renal excretion of digoxin. The dosage of digoxin, when changing preparations, should be adjusted individually.

Comparisons based on acute absorption tests, often with healthy subjects, have indicated differences between various digoxin preparations (17). There are, however, various opinions about the clinical significance of these differences in bioavailability. Clinically the most important changes are those which take place during maintenance therapy when a preparation is being replaced by another. Manninen et al. (18) showed that when they replaced one preparation by another therapeutically significant changes took place.

Digoxin is eliminated mainly unchanged by renal excretion. It is therefore possible that during maintenance therapy the 24-hour urinary excretion of digoxin will reflect more reliably the total amount of digoxin absorbed than does the concentration in the serum. We have therefore compared three digoxin preparations, administered to the same group of patients during maintenance therapy by measuring the concentration of digoxin both in the serum and in the urine. The group we chose consisted of aged patients expected to be most susceptible to any changes that may take place.

MATERIAL AND METHOD

The study was carried out in two stages on patients suffering from heart insufficiency in Turku City Hospital. The preparations compared were Digoxin (S) by Star used as basic drug for all the patients, Lanoxin Novum (W) by Wellcome, and Lanadix Novum (L) by Leiras (not yet launched on to the market) used as test preparation.

Stage 1. The rapidity of the change of the steady state serum digoxin level was studied in 25 patients (19 female and 6 male) of average age 70 years. All three preparations were compared in a cross-over manner on 13 patients, preparation S was compared only with one of the preparations W or L on 12 patients.

Digoxin was given orally once a day, either 0.250 or 0.375 mg, and no change in dosage was made during the study. The samples were collected 24 hours after the previous dose. A steady state concentration was determined on two consecutive days in patients taking preparation S and 1, 2 and 10–14 days after the change of preparation. Before changing over to the third preparation, the patients were again given preparation S for a fortnight.

Stage 2 was carried out on 11 aged patients (9 female and 2 male) with heart insufficiency. Their average age was 77 years (range 65–84). The serum digoxin levels of all the patients were compared after 10–14 days therapy on two consecutive days with each preparation. As in stage 1, serum sampling was carried out 24 hours after the previous digoxin dose. On the same days 24-hour urine was collected with indwelling catheter. On the test day an ECG was taken and the digoxin clearance was calculated by analysing the concentrations of 24-hour urine and of serum digoxin on the same day. Digoxin clearance was

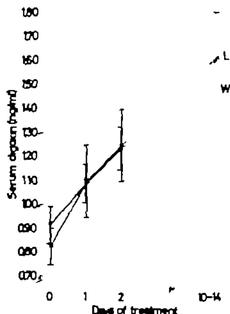


Fig. 1. Change of serum digoxin concentrations (±S.E.M.) after replacing preparation S (at 0) by L or W.

1.73 m² BSA. The 10–14 days therapy with different preparations was carried out by following the order S–W–S–L or S–L–S–W. The latter part of the study followed the principles of stage 1. Simultaneous use of diuretics or other drugs during the study was not restricted.

y of digoxin

roimmunological determination of digoxin in the and in the urine was always carried out in duplicate by using the method of Smith et al. (20). Digoxin was extracted twice from the urine with dichloromethane, evaporated to dryness and diluted into suitable amount of phosphate buffer solution for determination. The mean recovery of the method was 89%. The antiserum was prepared according to the method of Smith et al. (21) by immunizing rabbits with digoxin-protein conjugate.

RESULTS

In stage 1 we observed that the serum digoxin levels increased clearly when changing preparation S to either W or L (Fig. 1). The rapidity of this change was the same for both brands although individual differences were relatively great.

The mean steady state serum digoxin level (±S.E.M.) was 0.92±0.08 before changing preparation S to W. When S was changed to W respectively L, the corresponding figures were 1.48±0.12 and 0.83±0.08. Both changes were statistically highly significant ($p<0.001$).

The material and results of stage 2 are presented

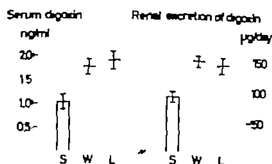


Fig. 2. Serum and 24-hour urinary digoxin levels (mean ±S.E.M.) in 11 patients treated with preparations S, W and L.

in Table I. Preparations W and L caused an increase both in the mean steady state serum levels and in the urinary excretion of digoxin when compared to preparation S. These changes were all statistically highly significant ($p<0.001$). The mean levels are illustrated in Fig. 2. It is seen that when a higher serum level is achieved there is an almost equal increase in the mean urinary excretion of digoxin. In one patient (no. 8), however, no increase in the serum digoxin levels was found when changing to the preparations with better absorption. The good absorption was totally compensated by an increase in the renal excretion of digoxin. The creatinine clearance of this patient was not reduced.

Creatinine clearance was somewhat decreased in several of these elderly patients. There appears to be a positive correlation between creatinine and digoxin clearances (Fig. 3). The measurements

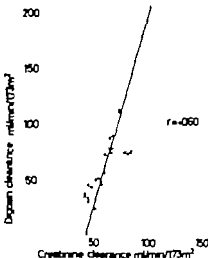


Fig. 3. Correlation between creatinine and digoxin clearances in 11 patients during maintenance therapy.

Table I *Steady state serum digoxin levels and daily urinary excretion of digoxin of 11 elderly patients*

Pat. no.	Age (y)	Se	Average creatinine clearance (ml/min/1 73 m²)	Daily digoxin dose (μg/d)	Daily digoxin dose (μg/kg)	Average steady state serum digoxin (ng/ml) S	W	L	Average urinary digoxin excretion (μg/d.) S	W	L	Average digoxin excretion (% of daily dose) S	W	L
1	70	♂	74	250	5 6	0.45	1.8	1 75	42.8	159.6	1[illegible]5 0	17	64	50
2	88	♀	40	250	4.2	2.0	2.65	2.75	78.3	161 0	120.9	31	64	48
3	83	♀	59	250	5.3	1 45	1 95	2.75	99 4	179 7	127 0	40	77	51
4	79	♀	46	250	4.5	1.3	2.65	2 0	97.[illegible]	148.0	116.0	39	59	47
5	79	♀	44	250	5 1	0.6	1 75	2.75	47.8	95 1	101 9	19	38	41
6	79	♀	54	250	4.5	1 2	1.43	1.85	76.7	130.9	141 7	31	52	57
7	75	♂	69	250	4 0	1 0	1 75	1.8	115 1	130.2	177 6	46	57	71
8	65	♀	82	375	4 9	1 15	1.25	1 15	109 4	177 1	259 0	29	47	69
9	70	♀	81	250	3.5	0.83	1 05	1.25	151 1	204.3	163 9	60	82	66
10	82	♀	65	250	6.3	1 13	1 95	1 75	102.8	156.5	131 9	41	63	53
11	84	♀	61	250	7 4	0.3	1.3	1.25	87.[illegible]	129.0	121.5	35	57	49
Mean	77		61	261	5 0	1 04	1 77	1 91	91.6	151 9	144.3	34	59	55
±S.E.M.			4	11	0.3	0.15	0.16	0.18	9.[illegible]	9 0	13.[illegible]	4	6	3

were carried out on two consecutive days in 11 patients during maintenance therapy with three different preparations. The correlation coefficient (r) was +0.60 and the correlation is highly significant ($p<0.001$). Digoxin clearance is on an average 73 ml/min/1 73 m² throughout the material and does not deviate significantly when measured separately during each therapy (S 76 W 74 L 69 ml/min/1 73 m²). Renal excretion was thus dependent on absorption and, in spite of decreased kidney function increased serum digoxin levels caused an increase of digoxin excretion into the urine

A comparison was made of the combined means of the serum digoxin levels in stages 1 and   The values obtained are shown in Table II The concentrations in individual patients during maintenance therapy are based on 2–4 determinations. Differences S–W and S–L are statistically highly significant ($p<0.001$) the difference W–L is not significant. Compared with preparation S the average mean obtained with W was 62% higher and with L 75% higher

DISCUSSION

Our study shows that when one digoxin preparation is changed to another with better absorption during maintenance therapy the improved absorption will be greatly compensated by increased excretion of digoxin into the urine also in all these elderly patients. Although none of our patients were uraemic and their serum creatinine levels were normal the average creatinine clearance was low When the steady state digoxin level in the serum with preparation W was on an average 70% and with L 84% higher than with preparation S the digoxin excretion into the urine was respectively 66 and 58% higher

As far as we know only in the study of Falch et al (1) has digoxin excretion into the urine been used in a comparison of bioavailability of digoxin. These investigators compared the new and old preparations of Lanoxin. In our study preparation W is the same new Lanoxin preparation as used by them. Falch et al found that 46% of the daily digoxin dose was excreted into the urine with this preparation during 4 hours, while our material showed 59% The patients of

Table II *Mean steady tate rum digoxin con- ce trations in th total material*

	Preparation S	W	L
Mean	0.98	1.59	1 77
No. of pats.	36	28	30
S.D.	0.38	0.51	0 75
S.E.M	0.06	0.10	0.14

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EFFECT OF NICOTINIC ACID ON MUSCLE LIPID CONCENTRATION IN MAN

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Abstract Muscle biopsies have been taken by needle biopsy from the femoral muscle of 1 subjects with hyperlipidemia before and during nicotinic acid treatment. N consistent changes were observed in the muscle triglyceride (TG) concentration during nicotinic acid treatment when all subjects were regarded as group. In subjects with high initial TG values in muscle (40.0–76.1 μmole/g, =5) however decrease and in subjects with initial low values (8 9–14.3 μmole/g, =7) an increase was observed during nicotinic acid treatment.

Nicotinic acid lowers the concentration of cholesterol and triglycerides (TG) in plasma (5 6) and has found use as a drug in the treatment of hyperlipidemia (3 7) Little information is however available on the effect of long-term administration of nicotinic acid on the lipid metabolism in tissues Carlson et al have previously observed reduced levels of TG in myocardium, skeletal muscle and liver in rats after acute administration of nicotinic acid (4) and there is evidence that nicotinic acid administration to hyperlipidemic subjects may lower the TG concentration also in femoral muscle (8). A report is here given on the concentration of cholesterol and TG in skeletal muscle of man during treatment with nicotinic acid

SUBJECTS

The subjects were obtained among participants in health survey study in Stockholm. Those with elevated levels of total plasma lipids were admitted to the clinic as outpatients for further investigation of their plasma lipid metabolism. Twelve of them 7 women and 5 men, volunteered for muscle biopsy and were given nicotinic acid (Nicangin® Draco Sweden), 1 5 g three times day In order to lower the plasma lipid concentration All subjects were in full-time work No advice with regard to plasma lipid lowering regimes had been given before nicotinic acid treatment

Table I gives some anthropometric data of the subjects and their types of hypertriglyceridemia (1) determined by paper electrophoresis and separation of the plasma lipoprotein fractions by ultracentrifugation before treatment. All had normal values with regard to concentration of glucose in blood, transaminases, alkaline phosphatases and uric acid in plasma.

METHODS

Sampling was done between 8 and 9 a.m. after overnight fast before and during nicotinic acid administration The concentration of TG and cholesterol in plasma was determined by the AutoAnalyzer technique (11) Muscle tissue was taken by needle biopsy from the vastus lateralis of the femoral muscle according to Bergström (2). The muscle tissue was dissected at magnification of 40 under dissection microscope to remove visible adipose tissue and connective tissue. After freeze-drying, the muscle material was divided into 7 pieces, separately extracted for lipids, and the concentration of TG and cholesterol was determined as previously described in detail (10). A mean value for the concentration of cholesterol and TG in each muscle sample was then calculated from the separate determinations and used in the tables. All muscle values are given on dry weight basis

Evaluated by analysis of ariance the S D for the TG concentration in muscle sample was found to be 5 μmole/g, for cholesterol 0 25 mg/g.

RESULTS

As evident from Table II muscle biopsies were taken with a mean interval of 6 1 and 16.3 weeks The mean values for the plasma TG concentration decreased consistently during nicotinic acid treatment. The cholesterol concentration decreased from mean of 337 to 56 mg/100 ml after 6 weeks of treatment and remained at this level with continued nicotinic acid administration. No significant changes were observed in the mean concentration of TG or cholesterol in muscle tis

Table I *Anthropometric data and types of hyperlipedemia*

	N	Age (y.)	Index[a]	Weight (kg)	Type[b] N	II A	II B	IV
Women	7	52.6±3.5	1 13±0.10	70 4±5.5	1	5	1	
Men	5	49 4±4.5	1 07±0.05	74 4±1 9	1	1	1	2
Total	1	51.3±_7	1 11±0.06	72.1±3 6				

$\frac{\text{weight}}{\text{height} - 100}$

According to Baumont et al. (1): N=normal lipoprotein pattern

sue at the different times of sampling when all subjects were regarded as one group (n=1)

The muscle TG concentration before nicotinic acid administration was 37 7±9 6 μmole/g in the women (range 75.8–14 3) and 26 7±1_6 μmole/g in the men (range 76.1±8 9) No correlation was found between the TG concentration in muscle and plasma (r=0 22, p>0.05)

Fig. 1 gives the individual percent change in the muscle TG concentration during nicotinic acid treatment. As evident from the Figure the change in the muscle TG concentration decreased in 5 and increased in 7 subjects. In the subjects with the marked initial increase of muscle TG concentration during nicotinic acid treatment the absolute values changed from 8 9 to 31 7 μmole/g _56%) and from __4 to 73_ μmole/g (_27%), respectively

When further analysed the decrease in the muscle TG concentration was observed only in subjects with initially high values (range 76.1–40.5 μmole/g) and the increase only in subjects with low initial values (range 8.9–22.4 μmole/g). Calculated as absolute values the mean muscle TG concentration was reduced from a mean of 58 to 30 9 μmole/g after 6 weeks of treatment in subjects with initially high values while an increase was found from a mean of 15 to 30 9 μmole/g after a similar period of treatment in subjects with initially low values (Table III). Treatment for more than 6 weeks caused no further change in the muscle TG concentration The concentration of cholesterol in muscle remained unchanged during nicotinic acid treatment in the two groups arbitrarily formed on the basis of the muscle TG concentration (Table III)

DISCUSSION

Nicotinic acid administration lowered the plasma TG concentration in all subjects but had varying effects on the muscle TG concentration. With high initial values for the muscle TG concentration a decrease was observed, with low an increase Fröberg (8) has previously reported a positive relationship between the decrease and the initial muscle TG concentration in male hyperlipidemic subjects after a period of 6 weeks of nicotinic acid administration

The divergent effect of nicotinic acid treatment on the muscle TG concentration observed here suggests that the metabolism of muscle TG is not

Table II *Sample interval and concentration of TG in plasma (TGpl) and in muscle (TGm) and of cholesterol in plasma (Cholpl) and in muscle (Cholm) during nicotinic acid treatment*

	Sample no. 1		3	4
No. of subjects	1	11	10	4
Weeks	0	6.0±0.4	12 0±0 7	16.3±1 7
TGpl (mmole/l)	3 71±1 02	1.60±0.28	1 46±0.22	1 18±0.10
Cholpl (mg/100 ml)	337±9.8	256±15.8	_50±15	771±9
TGm (μmole/g)	33 1±7 1	31 7±5 4	28._±4.0	27.6±4.1
Cholm (mg/g)	1 96±0.10	2._4±0.17	1 89±0.17	1.80±0.15

Table III. *Muscle lipids during nicotinic acid treatment in subjects with initially high and with initially low values for the muscle TG concentration*

No. of subjects within parentheses. Abbreviations as in Table I

	Sample no.			
	1		3	4
TGm (μmole/g)				
High	58.2±7 4 (5)	33.2±8.9 (4)	35.6±11.3 (3)	–
Low	15.2±1.7 (7)	30.9±7.3 (7)	25.5±3.5 (7)	27 6±4 1 (4)
Cholm (mg/g)				
High	2.03±0.15	..52±0.44	1 90±0.34	–
Low	1.91±0.14	2.1"±0.16	1.89±0..1	1.80±0.15

directly related to the metabolism of plasma TG This is also in accordance with the lack of correlation between the concentration of TG in plasma and muscle previously observed in subjects with elevated plasma lipid levels (8). This is at variance with findings in men with normal levels of plasma TG 2.0–2 1 mmole/l, where a positive although weak correlation was found between the concentration of TG in plasma and muscle (9 10) Interestingly in healthy subjects (10) the mean value for the muscle TG concentration was 29 1± 1 3 μmole/g dry weight (n=47) a value of the same order of magnitude as was approached in the present study during nicotinic acid treatment.

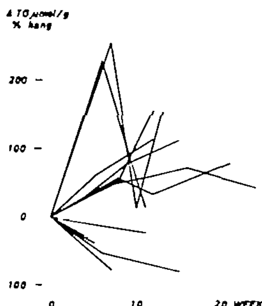


Fig 1 Percent change in muscle TG concentration during nicotinic acid treatment.

Studies on the metabolism of the muscle TG may reveal another facet of lipid metabolism which in clinical work is usually defined and evaluated on the basis of the pattern and metabolism of the plasma lipids. The different response observed in subjects with low and with high initial muscle TG concentration during nicotinic acid treatment does not argue against this concept. Further studies are however necessary to evaluate this aspect.

ACKNOWLEDGEMENTS

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DEMONSTRATION OF AN ENHANCED α-ADRENERGIC RESPONSE IN HUMAN ADIPOSE TISSUE WHEN INCUBATED IN PRESENCE OF Li^+ INSTEAD OF Na

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Abstract A study has been made of the effect of Li^+ on the adrenergic receptor response in human adipose tissue Subcutaneous adipose tissue was preincubated and incubated in media in which Na had been replaced by Li^+ The adrenergic response of the tissue was assayed by measuring the rate of lipolysis stimulated with noradrenaline in the presence or absence of phentolamine The capacity of the hormone-sensitive lipase was measured by the use of dibutyryl cyclic AMP The results showed that the α-adrenergic response was enhanced by the presence of 60 mM Li while the β-adrenergic response was affected only in a higher concentration of Li^+ (120 mM). The response of the lipase enzyme to dibutyryl cyclic AMP remained unaffected by Li however The effect of Li was inhibited by 0.5 mM La^{+++} in the medium indicating that Li^+ had increased the entry of Ca^{++} into the cells. These results suggest that the α-adrenergic response of human adipose tissue depends on the intracellular concentration of Ca^{++} for its inhibitory action on the adenyl cyclase enzyme

It has previously been demonstrated that the lipolytic response to noradrenaline in vitro is markedly diminished in adipose tissue from hypothyroid subjects. The decrease was shown to be due to an enhancement of the α-adrenergic receptor response of the tissue (19) which inhibited the formation of cyclic AMP (9). A similar increase in α-adrenergic response was also demonstrated in aortic smooth muscle from hypothyroid rabbits (18) How the lack of thyroid hormones renders the tissue more responsive to α-adrenergic stimulation is however not known at present It has been suggested that it might be the result of a change in the intracellular ion content (17) This hypothesis is supported by the demonstration that more Ca^{++} was liberated by α-adrenergic stimulation from intracellular pools in smooth muscle from hypothyroid than control strips (18).

The present study was undertaken in order to examine whether the α-adrenergic response in normal human adipose tissue could be enhanced by an increase in the intracellular concentration of calcium.

METHODS

Subcutaneous adipose tissue was obtained from subjects undergoing cholecystectomy None of the patients showed evidence of diabetes mellitus or other metabolic disease The biopsies were removed after induction of general anaesthesia by Nembutal® (pentobarbital sodium) nitrous oxide and Halothane® (bromochlorotrifluoroethane). The tissue was transported in 0 9% NaCl from the operating room and incubated as previously described (8).

The tissue was preincubated for one hour in Krebs-Henseleit bicarbonate buffer (KHB) (pH 7 4) containing 1% bovine albumin. Separate 50–100 mg pieces were then transferred to plastic vials containing 1.5 ml incubation medium made of KHB with 3% albumin, 1 mg/ml glucose (pH 7 4) The incubation were carried out at 37°C for two hours using air as the gas phase The release of glycerol was used as an index of lipolysis and analysed according to Chernick (5)

In experiments in which Li was used, NaCl was replaced by equal amounts of LiCl. In order to block the entry of extracellular Ca^{++} into the cells the rare earth metal lanthanum (La^{+++}) was added to the preincubation and incubation media. In this series of experiments the medium of van Breemen and McNaughton (4) was used instead of KHB since the presence of phosphate and bicarbonate in the KHB would have formed insoluble salts with La^{+++} The medium had the following composition. 160 mM NaCl or 160 mM LiCl, 4.6 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM Tris-HCl (pH 7 4). The same concentration of albumin and glucose were used in the preincubation and incubation media as in the experiments with KHB.

The following pharmacological agents were used in the incubations: l-noradrenaline bitartrate (Astra, Sw

Table 1 *Effect of different concentrations of Li⁺ in the preincubation media on basal lipolysis (mean±S.E. N=6)*

	Concentration in medium				p^a
Li (mM)	0	30	60	120	
Glycerol (μmoles/gww/2 h)	0.342±0.077	0.314±0.091	0.252±0.095	0.193±0.043	N.S.

Calculated from two-way analysis of variance (11).

den) phentolamine (Regitin Ciba, Switzerland) and N⁶ ⁰-dibutyryl cyclic 3',5'-adenosine monophosphate (Boehringer/Mannheim, West Germany).

Statistical calculations were performed on a PDP 8L computer using the FOCAL statistical routines of McKeown (13) or the two-way analysis of variance programme by Hall (11).

RESULTS

Replacing Na by Li in the preincubation and incubation medium did not significantly reduce the basal glycerol release from the tissue (Table I).

The effect of different concentrations of Li⁺ on the net lipolytic response to 2×10^{-6} M noradrenaline, $\times10^{-6}$ M noradrenaline plus 5 μg/ml phentolamine or 10^{-4} M dibutyryl cyclic AMP is shown in Fig. 1. Increasing the concentration of Li significantly and proportionately reduced the noradrenaline response, while the responses to noradrenaline plus phentolamine or dibutyryl cyclic AMP were not significantly modified.

The effect of 60 and 120 mM Li on the dose response to noradrenaline in the presence and absence of phentolamine is shown in Figs. 2 and 3. At a Li concentration of 60 mM the noradrenaline response was markedly reduced while that to noradrenaline plus 5 μg/ml phentolamine remained unaffected (Fig. 2). At a higher concentration of Li (120 mM) however the response both to noradrenaline and to noradrenaline plus 5 μg/ml phentolamine was reduced as shown in Fig. 3. Only the highest concentration of noradrenaline used (14×10^{-6} M) gave a significant stimulatory effect in the presence of phentolamine.

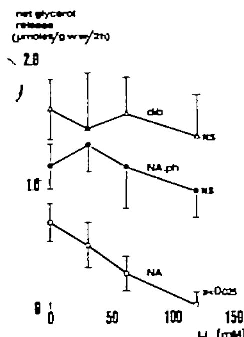


Fig. 1. Effect of different concentrations of Li in the preincubation and incubation media on the net lipolytic response to 10^{-4} M dibutyryl cyclic AMP (*dib*) (Δ—Δ), 2×10^{-6} M l-noradrenaline (*NA*) (○—○) and 2×10^{-6} M l-noradrenaline plus 5 μg/ml phentolamine (*NA.ph*) (●—●) (mean±S.E., N=6). The *p*-values were calculated from a one-way analysis of variance (11).

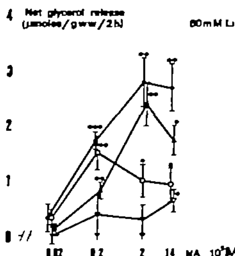


Fig. 2. Effect of 60 mM Li⁺ on the net lipolytic response to different concentrations of l-noradrenaline (*NA*) in presence (○—○ ●—●) and absence (Δ—Δ ▲—▲) of 5 μg/ml phentolamine (mean±S.E. *N* 5). Filled symbols denote the results obtained in presence of Li while the unfilled symbols show the results in the normal KHB buffer $p<0.05$ $p<0.01$ $p<$ 0.001

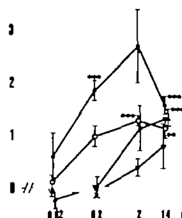


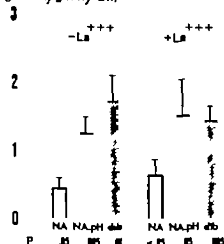
Fig. 3. Effect of 120 mM Li^{+} on the net lipolytic response to l-noradrenaline (*NA*) in presence and absence of 5 µg/ml phentolamine (mean±S.E. *N*=5). Symbols and *p*-values as in Fig. 2.

The presence of 0.5 mM La^{+++} in preincubation and incubation media modified the effect of Li on the adrenergic receptor response as shown in Fig. 4. When Na was replaced by Li in the medium the response to $\times 10^{-6}$ M noradrenaline was markedly reduced while that to 2×10^{-6} M noradrenaline plus 5 µg/ml phentolamine or dibutyryl cyclic AMP remained unaffected. The inhibitory effect of Li on the noradrenaline response was inhibited by the presence of 0.5 mM La^{+++}. The response to $\times 10^{-6}$ M noradrenaline plus 5 µg/ml phentolamine or 10^{-} M dibutyryl cyclic AMP was not affected by the presence of La^{+++}.

DISCUSSION

The present investigation was undertaken in order to examine whether a change of the intracellular ion content would affect the α-adrenergic response of human adipose tissue. The results show that the α-adrenergic response of the tissue could be selectively enhanced by substituting 60 mM Na for Li in the medium. The effect of Li was most probably due to an increased accumulation of Ca^{++} in the cells.

It has previously been demonstrated that calcium ions accumulate intracellularly if tissue specimens are incubated in sodium-deficient media. This has been shown for squid axons (1) smooth muscle (3) cardiac muscle (15) rat adipose tissue and brain tissue (6 20). From these studies it seems well established that the active transport of calcium out of these cells depends on a gradient of sodium over the cell membrane while the flow of Ca^{++} into the cells is passive. Li choline and sucrose do not participate in this sodium–calcium exchange and hence calcium will accumulate when sodium is replaced by any of these substances. So far no evidence of such a sodium–calcium exchange mechanism has been demonstrated in hu



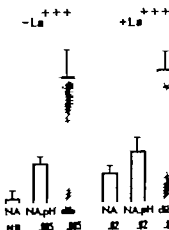


Fig. 4. Inhibitory action of 0.5 mM La^{+++} on the effect of Li-containing media on the net lipolytic response to $\times 10^{-6}$ M l-noradrenaline (*NA*), 2×10^{-6} M l-noradrenaline plus 5 µg/ml phentolamine (*NA,ph*) and 10^{-3} M dibutyryl cyclic AMP (*dib*) (mean±S.E. *N*=6).

man adipose tissue. However, in view of the many tissues which have been shown to possess such a sodium–calcium exchange mechanism a sodium-deficient medium was tried as an instrument to increase the intracellular concentration of calcium in the present series of experiments. Although no direct measurements of the Ca^{++} uptake were made, the finding that La^{+++} could antagonize the effect of Li^+ favours the presence of such a sodium–calcium exchange mechanism in human adipose tissue. This conclusion rests on the observations that La^{++} blocks the entry of Ca^{++} into cells by adhering to a cellular surface component which normally binds Ca^{++} (4). The sodium–potassium exchange, on the other hand, does not seem to be blocked by La^{+++} (1).

The replacement of Na^+ by Li^+ in the present study diminished the noradrenaline–stimulated lipolysis. The degree of inhibition depended on the concentration of Li^+ (Fig. 1). The lipolytic effects of noradrenaline plus phentolamine or dibutyryl cyclic AMP were, however, not significantly changed unless the concentration of Li^+ was high (120 mM) (Figs. 1 and 3). The effect of 60 mM Li^+ on the noradrenaline response and the absence of an effect on the response to noradrenaline plus phentolamine indicate that the α-adrenergic response was preferentially influenced at this concentration of Li^+. At a higher concentration of Li^+ (120 mM), however, both the responses to noradrenaline and noradrenaline plus phentolamine were affected, showing that the α- as well as the β-adrenergic responses were inhibited.

The present finding that the lipolytic effect of dibutyryl cyclic AMP was unaffected by Li^+ demonstrates that the effect of Li^+ does not set in at the level of lipase activation but at an earlier step in the lipolytic mechanism. Such a site could probably be the adenyl cyclase, since our previous studies have shown that the α-receptor acts by inhibiting the formation of cyclic AMP (10). The effect of Li^+ could be the result of either an increased intracellular accumulation of Ca^{++} due to the sodium-deficient media, to an inhibitory effect of Li^+ per se, or to a decreased intracellular concentration of K^+. Both Li^+ and Ca^{++} are known to inhibit adenyl cyclase, while K^+ stimulates the enzyme system (2, 7). It was of importance to differentiate between these possibilities. In order to study whether the effect of Li^+ was due to the entry of Ca^{++} into the cells, La^{+++} was used as an inhibitor of Ca^{++} influx. The concentration of La^{+++} (0.5 mM) used completely blocked the influx of Ca^{++} into K^+-depolarized smooth muscle as previously shown (18). In the present experiments the effect of Li^+ on the α-adrenergic response was counteracted by the presence of La^{+++} in the medium (Fig. 4). This indicates that the effect of Li^+ on the α-adrenergic response can be attributed to the entry of calcium into the cells and not of Li^+ per se. Nor could the effect of Li^+ be due to a decrease in K^+, since the β-adrenergic response elicited by noradrenaline in the presence of phentolamine was unchanged while the α-response was enhanced in the presence of 60 mM Li^+.

In human adipose tissue α-adrenergic stimulation inhibits the formation of c AMP and lipolysis, while β-adrenergic agonists stimulate the synthesis of c AMP and lipolysis (8, 10). The response to noradrenaline, a combined α- and β-adrenergic agonist, will therefore depend on the relative responsiveness of the two types of receptors in the adipocytes. The α-adrenergic receptor response can be modulated by the level of thyroid hormone. Thus specimens of adipose tissue from hypothyroid subjects showed a marked increase in the α-adrenergic responsiveness which completely inhibited the normal lipolytic effect to noradrenaline (19). The β-adrenergic response as measured by stimulation with isopropyl noradrenaline or noradrenaline in the presence of phentolamine, however, remained intact, as did also the hormone sensitive lipase enzyme (19).

The mechanisms behind the increase in α-adrenergic response in adipose tissue from hypothyroid subjects is not known at present. Earlier studies, however, have demonstrated that the hypothyroid state in human subjects is accompanied by an increased intracellular concentration of sodium (1). The underlying mechanism could possibly be a decreased Na^+–K^+ ATPase activity of the cells as demonstrated in hypothyroid rats (1). Such an enhanced intracellular concentration of Na^+ might in turn increase the concentration of Ca^{++} in the cells (17). Since the Ca_i^{++} was higher in smooth muscles from hypothyroid rabbits than controls upon α-adrenergic stimulation (18) it was postulated that the enhanced α-adrenergic response observed in human adipose tissue from hypothyroid subjects could also be due to such a derangement of the intracellular Ca^{++} ion content

(17). The present finding that Li^{+} could increase the α-adrenergic response and that the effect of Li^{+} was blocked by the presence of La^{+++} favours the hypothesis that calcium is involved in the α-adrenergic response in human adipose tissue. Furthermore, since it was shown previously that the difference in noradrenaline response between normal adipose tissue and that from hypothyroid subjects was due to a quantitative rather than qualitative change in adrenergic response (16), it might be postulated that the observed enhancement of the α-response in tissues from hypothyroid subjects could also be due to an increased concentration of Ca_i^{++} upon α-adrenergic stimulation. In this context it is of interest to note that ouabain, which blocks the Na^{+}-K^{+} ATPase, inhibited the lipolytic response to noradrenaline in the human forearm preparation in healthy subjects (14).

The present study has thus shown that the α-adrenergic response in human adipose tissue can be enhanced in vitro by the presence of Li^{+} in the incubation media. The change induced by Li^{+} was most probably the result of an increased accumulation of calcium intracellularly, since it could be antagonized by La^{+++}. Whether α-stimulation is expressed through an increase in ionized calcium intracellularly or whether it increases the affinity of Ca^{++} for the adenyl cyclase, however, cannot be determined from the present study.

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PROTEINURIA IN NON RENAL INFECTIOUS DISEASES

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Abstract. The urinary excretion of total protein, orosomucoid, albumin, transferrin, and IgG, has been determined in 19 children and 23 adult with various non-renal infectious diseases. Furthermore urine muramidase was estimated in 39 of these patients (16 children 23 adults). An often considerably increased urinary protein excretion ("febrile proteinuria") was found in 74% of the children with maximal output of 2283 mg/24 h and in 83% of the adults, maximal output 3350 mg/24 h. In all cases an increased excretion of orosomucoid was found, but in many cases the excretion of the other proteins determined was increased as well, which excludes prerenal mechanism as the only factor responsible for the increased protein excretion. The hypothesis is put forward that infections result in an immunological injury of the glomerular and/or tubular basement membrane, which might be of pathogenetic importance in the development of renal diseases.

Different factors among them fever influence the normal urinary protein excretion. The term "febrile proteinuria" is used extensively but only a few investigations concerning this subject have been published. Welty (24) examined the urinary protein excretion in 40 patients undergoing fever therapy In 78% of the cases a substantial increase in the protein excretion was found. Ehrström (5) demonstrated proteinuria in 36% of 300 patients with different infectious diseases. The highest output of protein was found in patients with pneumococcus and influenza infections. Marks et al (12) investigated urine protein in 198 children with febrile illnesses Proteinuria was found in 5.6%.

Thus earlier investigations have in most cases determined the urinary excretion of total protein, and the results of these determinations have been somewhat divergent. Specific immunochemical methods for quantitation of different plasma proteins are now available Therefore we examined the urinary excretion of total protein and 5 plasma proteins in 42 patients suffering from various non-renal infectious diseases.

MATERIAL

The patients were admitted to an emergency section of the University Clinic for Infectious Diseases, Copenhagen. For practical reasons only one patient per day the first with rectal temperature ≥38°C on admission, was chosen. To enter the study the patients so selected should fulfil the following criteria. 1) No kidney diseases in the family N previous or present kidney disease or generalized disease with known probable kidney affection. 2) Normal urine sediment by microscopy and no growth of bacteria in the rine.

The material comprised 42 patients, 11 females and 31 males aged 2 3/4–76 years, 19≤15 years Details of the patients are given in Table I.

The control material comprised 12 adult persons from the laboratory and medical staff, 7 females and 5 males aged 25–69 years. Nobody had previous kidney diseases or signs of present infectious diseases. Their Hb concentration, ESR, serum paper electrophoresis and serum creatinine were normal. The urinary sediments were normal and there was no bacterial growth in the urines.

METHODS

The patients urine was quantitatively collected from 12 p.m. after admission and during the following 4 hours. In one patient (no. 24) 17 consecutive 24 h portions of urine were collected.

During collection the urines were stored at 4°C and sodium azide was added as preservative to give final concentration of 0.1% (w/v). During collection of urine the patients were confined to bed. In the middle of the collection period blood sample was drawn and from this serum was used Mean temperature signifies the temperature at the start of the collection period plus the temperature at the termination of collection period divided by 2.

Immediately after admission material from the infected organ was obtained for microscopy and cultivation and appropriate antibiotic therapy was instituted.

Table I *Clinical and laboratory data of 42 patients with various infectious diseases*
NI = not identified

Pat. no	Sex	Age (y)	Diagnosis	Infecting micro-organism	Mean temp. (°C)	Cl_{Cr} (ml/min)	Albustix® reaction	Urine protein (mg/24 h)	Agarose-gel pattern[a]
1	♂	2 3/4	Bact. meningitis	Neiss. mening.	37 0	15	–	117	B
2	♂	4	Lymph. meningitis	NI	38.5	37	Trace	264	C
3	♂	4	Lymph meningitis	Mumps virus	39.5	0.7**	Trace	143	B
4	♂	5	Bact. meningitis	Haem. influen.	36.6	22	Trace	1 232	E
5	♂	6	Tonsillitis	NI	38.8	0.7**	–	65	A
6	♂	6	Bact. meningitis	NI	38.	2.03*	–	383	C
7	♀	6	Pharyngitis	NI	39 0	66	Trace	91	A
8	♂	8	Tonsillitis	Haem. haemolyt.	37 1	66	Trace	56_	E
9	♀	8	Pharyngitis	Haem haemolyt.	37 9	23	–	63	A
10	♂	9	Lymph. meningitis	Mumps rus	37.8	31	Trace	97	A
11	♂	9	Inf mononucleos.		38.8	97	Trace	.30	A
1	♂	10	Lymph. meningitis	Mumps viru	39.6	49	–	151	B
13	♂	10	Pneumonia	NI	38.3	89	Trace	138	B
14	♀	11	Malaria	Plasm vivax	38 7	57	Trace	47	A
15	♂	11	Lymph. meningitis	Mumps virus	38.5	66	–	167	A
16	♂	13	Pneumonia	NI	39.6	44	Trace	490	D
17	♀	13	Pneumonia	Pneumococcus	38.2	83	+	1 910	E
18	♂	15	Febris causa ign.	NI	38.8	158	–	401	B
19	♂	15	Pneumonia	Pneumococcus	37.8	105	+	2 283	C
20	♂	16	Sepsis	NI	39 0	103	Trace	367	B
21	♂	16	Lymph. meningitis	NI	38.5	156	+	366	B
22	♂	17	Bact. meningitis	Neiss. mening.	37.8	150	Trace	784	C
23	♂	2_	Tonsillitis	NI	39 4	118	+	708	C
4	♂	22	Bact. meningitis	Neiss. mening.	39 7	165	+	2 687	C
25	♀	25	Lymph meningitis	NI	38.1	72	Trace	426	D
26	♂	26	Malaria	Plasm. vivax	38 9	61	Trace	390	C
27	♂	26	Mumps	Mumps virus	37.5	108	Trace	1 360	D
28	♂	32	Acute gastroent	Salm. sero-typ. kumgi	37 7	33	Trace	30	A
	♂	33	Postvacc. encephalit.		38.5	52	–	1 1	A
	♂	34	Impetigo	Haem strept.	37 7	135	+	750	C
)	♂	42	Mumps	Mumps virus	40.6	97	+	1 690	E
	♂	42	Bact. meningitis	Pneumococcus	38.5	105	++	3 350	E
	♀	43	Acute gastroent.	Salm. typ murium	38 7	66	+	486	C
34	♂	44	Acute gastroent.	NI	38.6	84	Trace	1 212	C
35	♂	49	Bact. meningitis	Pneumococcus	39.3	90	Trace	1 333	D
36	♀	50	Febris causa ign.	NI	37.6	84	–	113	A
37	♀	56	Pharyngitis	Haem influen.	38.1	94	Trace	95	A
38	♂	60	Bact. meningitis	Pneumococcus	38.5	66	+	2 180	E
39	♀	6_	Bact. meningitis	Pneumococcus	37 7	84	Trace	694	F
40	♂	67	Malaria	Plasm. malariae Plasm. falc	38.9	2.74*	++	530	D
41	♀	73	Pneumonia	NI	38 6	101	+	1 010	E
42	♀	76	Bact. meningitis	NI	38.7	49	Trace	304	F

Serum creatinine (mg/100 ml).
A = normal B = broad α and fast α_2, C = tubular D = glomerular E = glomerulo-tubular F = unclassifiable

LABORATORY METHODS

The 24 h portions of urine were tested by means of Albustix® and aliquots were withdrawn for the following examinations. 1) Protein. A biuret method was used, slightly modified after Sa ory et al. (17) 2) Microscopy cultivation and determination of creatinine

The residue of the 24 h portions was concentrated at 4°C by means of low pressure *ultrafiltration* using Visking dialysis tubes 23/32 inches (6). In the concentrated urines and in the serum samples the following proteins were quantitated by means of immunoelectrophoresis in antibody-containing agarose gel: orosomucoid, albumin, transferrin, IgG and muramidase (11, 22, 23) The antibodies to the different proteins were obtained from Dakopatts A/S Copenhagen.

In order to evaluate the type of proteinuria, agarose-gel electrophoresis was performed in 0.075 M veronal buffer of *pH 8.4* containing *1% (w/v) agarose*. The electrophoresis was run for 45–60 min at 20 V/cm.

Table II *Proteinuria serum concentration and urinary excretion (mean and observed range) of 5 plasma proteins in 12 controls*

	Total protein	Muramidase	Orosomucoid	Albumin	Transferrin	IgG
Serum (g/l)	–	_6[a] 1.3–3 0	0.78 0.67 1 00	38.2 31 7–45.	_28 1.80–3 06	I__ 7 3–19.8
Urine (mg/24 h)	82 43–123	51[b] 17 100	0 42 0 1 –0 82	7 7 3 9–15 0	0 18 0.06–0.36	2.8 1 0–4.3

mg/l. μg/24 h

RESULTS

Controls

The urinary protein excretion, the serum concentration and the urinary excretion of muramidase orosomucoid albumin, transferrin and IgG in the 12 control subjects are shown in Table II The protein excretion was 43–123 mg/24 h. The mean urinary excretion of orosomucoid albumin, transferrin and IgG was 0.4? 7 7 0.18 and _8 mg/24 h, respectively Urine muramidase was on an average 51 μg/24 h.

Patients

Albustix reaction. In 9 cases the Albustix reaction was negative in 1 it showed a trace in 10 cases + and in cases ++ In the 9 cases with negative Albustix reaction the urinary protein excretion varied from 52 to 401 mg/24 h in the 1 cases with trace reaction from 30 to 1 369 and in the 10 cases with +reaction from 366 to 2 687 In the 2 patients with ++reaction, the proteinuria was 530 and 3 350 mg/24 h respectively

Urinary protein excretion In the 19 patients ≤15 years the urine protein was 63– 283 mg/24 h. In 14 cases it was above 123 mg/24 h (=highest value in the controls) In the 23 patients >15 years urine protein was 30–3 350 mg/24 h, and in 19 cases it was increased above normal. In 17 patients with a mean temperature <38.5°C the mean urinary excretion of protein was 649 mg/24 h. In the remaining 25 patients with a mean temperature ≥38.5°C the figure was 757 mg/24 h.

In 13 of the 19 patients ≤15 years the infection could with certainty be characterized as bacterial in 7 and viral in 6. In the 23 patients >15 years it was possible to characterize the infection as caused by bacteria in 12 and by virus in 5 The 7 children with bacterial infection had an average proteinuria of 936 mg/24 h (63–2 283) and the 6 with virus infection 175 mg/24 h (97–264) In the I adult patients with bacterial infection the proteinuria was on an average 1 088 mg/24 h (30–3 350) and in the 5 adults with viral infection 793 mg/24 h (1.1–1 690) Table III shows the mean and range of proteinuria in relation to the infecting microorganism.

In patient 4 a 22 year-old man with meningococcal meningitis, the daily urinary excretion of total protein, orosomucoid albumin, transferrin and IgG was followed for 17 days. The results are shown in Fig. 1

Table III *Proteinuria versus infecting microorganism*

C=children, A=adults

Microorganism		No. of pats.	Proteinuria (mg/24 h) Mean	Range
Pneumococcus	C		097	1 910– 283
	A	4	2 139	694–3 350
Meningococcus	C	1	117	–
	A		1 736	784– 687
Haemophilus influenzae	C	1	1 232	–
	A	1	95	
Haemophilus haemolyticus	C		313	63– 56_
	A	–	–	–
β-haemolytic streptococcus	C	–	–	–
	A	1	790	
Other bacteria	C	1	383	–
	A	4	297	30– 486
Mumps virus	C	4	140	97– 167
	A	2	1 525	1 360–1 690
Other virus	C		247	230– 264
	A	3	304	121– 426
Malaria	C	1	47	–
	A		460	390– 530

Table IV *Serum concentration and urinary excretion of 4 plasma proteins in 42 patients with infectious diseases*

D=decreased N=normal, I=increased C=children A=adult
Figures within parentheses=% of total

		No of pats											
		Orosomucoid			Albumi			Transferrin			IgG		
		D	N	I	D	N	I	D	N	I	D	N	I
Serum concentration (g/l)	C	1	1	17	4	12	3	7	1	0	4	15	0
		(6)	(6)	(88)	(21)	(63)	(16)	(37)	(63)	(0)	(21)	(79)	(0)
	A	1		20	4	18	1	13	10	0	3	20	0
		(4)	(9)	(87)	(18)	(78)	(4)	(56)	(44)	(0)	(13)	(87)	(0)
Urine excretion (mg/24 h)	C	0	0	19	0	9	10	0	4	15	0	4	15
		(0)	(0)	(100)	(0)	(47)	(53)	(0)	(21)	(79)	(0)	(21)	(79)
	A	0	0	23	1	4	18	0	4	19	1	2	20
		(0)	(0)	(100)	(4)	(17)	(79)	(0)	(17)	(83)	(4)	(9)	(87)

Orosomucoid In the children the urinary excretion was 4–855 and in the adults 5–472 mg/24 h.

Albumin In the patients ≤15 years the urinary excretion varied from 9 to 410 mg/24 h and in the patients >15 years from 2 to 1 054 mg/24 h

Transferrin In the two groups the urinary excretion was 0 10–32 and 0 10–74 mg/24 h respectively

IgG In the group ≤15 years the urinary excre was 1 2 94 and in the group >15 years 0.8–5 mg/24 h

Urine concentration mg/1000 ml
log to % of normal mean

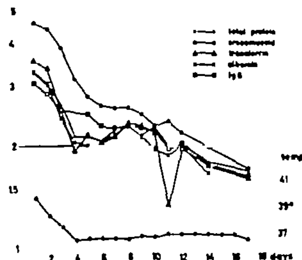


Fig 1 Urinary excretion of total protein and 4 plasma proteins in a patient with meningococcal meningitis

The results of orosomucoid albumin transferrin and IgG determinations are summarized in Table IV

Muramidase Serum and urine muramidase was determined in 39 (16 children 23 adults) of the 42 patients An increased urinary muramidase excretion (>100 μg/24 h) was found in 9 cases, 121–3 900 μg/24 h Three of these 9 cases were ≤15 years. In 7 of the 9 cases the infecting microorganism was identified. pneumococcus 4 cases, haemophilus influenzae 1 mumps virus 1 and combined infection with plasmodium malariae and plasmodium falciparum 1 case (Table V)

Agarose-gel electrophoresis of the urines

By means of agarose-gel electrophoresis of the urines it was possible to differentiate between five patterns of proteinuria A=normal (n=11) B=normal except for a broad α_1-band (presumably orosomucoid) and a distinct fast α_2-band (n=7) C=tubular pattern (n=10) D=glomerular pattern (n=5), E=mixed glomerulo-tubular (n=7) and F=unclassifiable (n=2) All the urines with patterns C, D and E except one also showed pattern B. Fig 2 shows the different patterns.

Thus an abnormal pattern was found in 73% of the urines examined Among the children 12 urines (63%) belonged to the first two patterns 7 (37%) to the last three while among the adults 6 (26%) had the first two patterns, and 17 (74%) the last three (Table I)

Table V *Data of 9 patients with muramidasuria*
NI = not identified

Pat no.	Diagnosis	Infecting microorganism	Creatinine clearance (ml/min)	Leukocytes /µl	Proteinuria (mg/24 h)	Muramidase Serum (mg/l)	Muramidase Urine (µg/24 h)
4	Bact. mening.	Haem. influen.	22		1 _3	_0	160
17	Pneumonia	Pneumococcus	83	11 400	1 910	3 7	2 010
19	Pneumonia	Pneumococcus	105	25 600	283	4.5	2 700
31	Mumps	Mumps virus	97	11 200	1 690	4 7	1 130
32	Bact. mening.	Pneumococcus	105	13 600	3 390	3 9	3 900
34	Acute gastroent.	NI	84	7 800	1 1	6 3	496
35	Bact. mening.	Pneumococcus	90	15 900	1 333	_1	305
40	Malaria	Plasm malariae Plasm. falc	_74*	10 800	530	6.6	650
41	Pneumonia	NI	101	–	1 010	3.3	121

Serum creatinine (mg/100 ml)

DISCUSSION

In the present study an adult control material was used both for children and adults. However as can be seen in Table I, a lowering of the upper limit of protein excretion in the group ≤15 years due to the supposedly lower normal protein excretion in children compared with adults (8) will cause only minimal elevation of the percentage of "febrile proteinuria" in children, as the urinary protein excretion in this group was either normal or markedly elevated The results obtained in the present control material are in accordance with those of earlier investigators (2 3 16)

In the patients proteinuria (>123 mg/24 h) was found in 74% of the children and in 83% of the adults Ehrström (5) found "febrile proteinuria" in only 36% But it is difficult to compare his results with those from the present study since the method of Esbach used by Ehrström gives variable results and probably excludes the more moderate "febrile proteinurias" Marks et al (12) found "febrile proteinuria" in only 5 6% of feverish children. This low percentage can in part be explained by their mode of selection which was based on an Albustix reaction >30 mg/100 ml corresponding to ++ or more in the present study But Albustix reacts poorly with orosomucoid and immunoglobulins If this method had been used in the present study 30 patients would have been excluded resulting in a lowering of the percentage of patients with "febrile proteinuria" to 29 Marks et al (12) found a positive Albustix reaction (as defined above) in 37 of 198 patients but only in 11 of these 37 cases was the sulphosalicylic acid test positive. Therefore they stressed the importance of a high incidence of false positive results obtained by means of Albustix but, as mentioned above they did not take into account the false negative reactions which are equally common.

Agarose-gel electrophoresis of the urines of 9 patients with normal urinary protein excretion revealed a normal pattern in 8 whereas in 1 patient the pattern was that of a broad α_1-band and an accentuated α_2-band. In contrast 3 patients with an increased protein excretion showed a normal pattern of agarose-gel electrophoresis. Thus in most cases a quantitatively abnormal protein ex

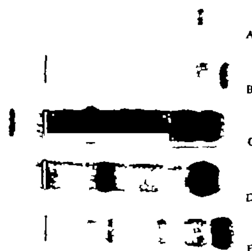


Fig 2 Different patterns of proteinuria obtained by means of agarose-gel electrophoresis.

cretion corresponded to a qualitatively abnormal one

In the children the proteinuria was greater in patients with bacterial than in those with viral infections This difference was not found in the adults. Among the bacterial infections those caused by pneumococci showed the greatest proteinuria. These findings are identical with those of Ehrström (5). Also mumps infection in adults was accompanied by a substantial rise in protein excretion

It has earlier been claimed that "febrile proteinuria" is almost exclusively prerenal (9) In the present study an augmented excretion of orosomucoid was found in 100% of the patients but in most cases the urinary excretion of albumin transferrin and IgG was also increased The augmented excretion of orosomucoid could be explained by a prerenal mechanism as the serum concentration of this protein was increased in most cases

The antiserum used to quantitate IgG was specifically directed to the H chain of IgG Consequently this antiserum reacts with free H chains or fragments of H chains Vaughan et al (20) found that about 15% of γ-globulins in urine from ...d persons represented the Fc fragment of ... In the present study it is most probable that part of this fraction designated IgG represents Fc fragments of IgG which owing to their lower molecular weight could cause a prerenal proteinuria However the increased excretion of albumin and transferrin indicates a renal (glomerular or tubular) lesion.

How far the febrile illness results in a glomerular and/or tubular injury was evaluated by measuring the urinary excretion of muramidase and by means of agarose-gel electrophoresis performed on the urines

At normal serum concentration an increased urinary excretion of muramidase is a sign of lesion of the proximal tubules (1 15) In the present study muramidasuria was found in 9 of 39 cases investigated As the serum concentration of muramidase in no case exceeded the renal threshold for muramidase the finding indicates proximal tubular damage The febrile illness in the 9 patients was caused by bacteria as well as by virus and malaria parasites. In 4 cases the infection was caused by pneumococci and in these cases the greatest muramidasuria was found

Of the 9 patients with muramidasuria agarose-gel electrophoresis showed a tubular or glomerulo-tubular pattern in 7 In the remaining 2 the pattern was glomerular Of the 15 patients with tubular or glomerulo-tubular pattern only 7 had muramidasuria Thus the correlation between these investigations was poor Which if any of the above mentioned methods permits the diagnosis of tubular damage cannot be evaluated from the present study

The renal lesion caused by an infectious disease is probably transitory In only 1 patient was the daily urinary excretion of different proteins determined for a lengthy period—17 days The result of this investigation showed that the urinary excretion of different proteins was not normalized until the 10th–14th day whereas the temperature was normal on day 4

Based on the above mentioned findings it can be concluded that an infectious disease in many cases results in a lesion of the glomeruli and/or tubules. The mechanism for this injury is unknown. A possible pathogenic factor is the elevated body temperature accompanying the infectious disease. Thus Welty (24) found an increased urine protein excretion in 78% of 40 patients in whom fever was induced by means of the Kettering hypertherm Ehrström (5) showed that the height and duration of the fever did not influence the magnitude of proteinuria in patients with infectious diseases whereas Marks et al. (12) found proteinuria only in patients with a body temperature above 38 4°C The results in the present study did not favour the view of body temperature as the only factor of importance for the augmented protein excretion in the urine Thus no difference in average proteinuria was found in 17 patients with a mean temperature <38.5°C nor in 25 patients with a mean temperature ≥38.5°C Furthermore in the patient in whom proteinuria was followed for 17 days the protein excretion did not become normalized until several days after the body temperature (Fig 1)

Since fever alone cannot explain the "febrile proteinuria" one might propose renal damage by bacteria or virus possibly by means of an immunological mechanism. One may advance the hypothesis that all infections result in the formation of antigen–antibody complexes in which the antigen is the infecting microorganism or parts of it and the antibody consists of immunoglobulins

produced by the host against the antigens. These complexes are deposited on the epithelial side of the basement membrane and result in lesion of the membrane How far this lesion results in a transient (febrile proteinuria) or more permanent (glomerulonephritis) abnormality depends, among other things, on the phagocytic function of the mesangium. If this function is undisturbed the immune complexes will be taken up and destroyed by mesangial cells and the result will be no or only a transient glomerular lesion If however the function of the mesangial cells is disturbed the immune complexes remain on the basement membrane resulting in more severe damage to the membrane with ensuing glomerulonephritis (13) According to this hypothesis one could imagine how an infection via bacterial or viral metabolic products temporarily poisons the mesangial cells with resulting febrile proteinuria" Recently it has been shown in experiments on rabbits that tubular damage as well may be caused by antigen-antibody complexes (10)

Whether infections result in deposition of immune complexes on the glomerular and/or tubular basement membrane can be evaluated by means of immunofluorescent microscopy on kidney biopsies obtained from patients with infectious diseases. Ward and Kibukamusoke (21) demonstrated malarial antigen as well as immunoglobulins and complement in kidney biopsies from patients with quartan malaria and the nephrotic syndrome and Combes et al (4) demonstrated the deposition of Australia antigen IgG and complement (C 3) in a granular pattern in the glomeruli of a patient with persistent Australia antigen in the blood and a membranous glomerulonephritis. Furthermore in patients *with infected ventriculoatrial shunt for* hydrocephalus and with glomerulonephritis immunofluorescent microscopy of kidney biopsy has demonstrated the deposition of immunoglobulins and complement in the glomeruli (19)

Before the days of immunofluorescent microscopy investigations clearly demonstrated structural and functional renal abnormalities in patients with infectious mononucleosis hepatitis and pneumonia of different etiologies (7 14 18)

The etiology in most cases of glomerulonephritis is unknown. On the basis of the above mentioned investigations including the results of the present study it seems reasonable to propose that infectious diseases of different etiologies might be of pathogenetic importance in the development of glomerulopathies

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

X Decremental Nerve Conduction in Uremia

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Abstract In uremic patient the sensory conduction velocity was relatively more reduced in the wrist–elbow segment than in the digit–wrist segment of the median nerv when the nervous impulse was initiated by supramaximal stimuli deli ered to sensory fibers in digit 1 or 3 The proximal segment became affected earlier in the course of progressive kidney insufficiency than the distal segment. These findings were also p- parent from the longitudinal course in the indi idual patient, and it is suggested that the uremic intoxica tion of the axon membrane function lead to decre mental impulse propagation

In a recent in estigation it was shown that action potentials become conducted with decrement when peripheral nerves in normal persons are subjected to ischemia (1) The sequence of changes in the nerve conduction during 30 min of ischemia was principally similar to that observed over months or years during progressive chronic renal failure (CRF) (10). This supports the hypothesis previously advanced (10) that, in addition to degenerative nerve lesions slowing of the nerve conduc tion in uremia also results from a toxic impair ment of the axon membrane function. The latter is inferred from other biophysical observations indicating that the Na –K activated ATPase is inhibited by dialysable humoral factor(s) n uremic serum (13) that the ntracellular sodium concentration is elevated (1 2) and that the resting membrane potential difference is reduced (3 5) The present report brings evidence indi cating that uremic intoxication leads to a decremental nerve conduction in analogy with the observations during schemia and in keeping w th the prediction by Lorente de Nó and Condouris (8)

Part of this study was presented at the VIII International Congress of Electroencephalography and Clinical Neurophysiology Marseilles, 1973 (11)

MATERIAL AND METHODS

A total of 67 patient with CRF was xamined on one or more occasions Their age ranged from 15 to 61 years. The kidney function was pressed as the average 24-hour endogenous creatinine clearance (C_{cr} ml/min) for three consecutiv day and corrected to body surface area of 1 73 m²

Thi analysis was confined to the distal and proximal segments of sensory fibers in the median nerv hereby voiding two sources of error (*a*) The conduction pattern resulting from generalized toxic membrane dysfunction might become obscured by degenerat nerv lesions, hich are most prominent in the legs and predominantly affect the most distal part of the nerv (6) (*b*) I motor nerves an increase the distal motor latency might reflect slowing of the conduction loc ity the terminal segment as well as an increased delay over the motor end plate, whereas the conduc tion elocity in more proximal segment is only deter mined by the latency difference between tw sites of stimulation.

Sensory action potentials were evoked by supramaximal stimulation of the nerv in digit 1 =141) or digit 3 (*n*=85) and recorded *simultaneously* through needle lectrodes placed close to the nerve t the wrist and the elbow (4) The action potentials were displayed on 3-channel electromyograph (DISA) The tempera ture near the nerve was about 35°C at both wrist and elbow Other details about the procedure and mean alues in normal material with the same age and se distribution ha been eported previously (9)

RESULTS

The regression (least square method) between con duction elocities in the digit–wrist (*Y*) and the wrist–elbow (*X*) segments had a slope of 0.55 and

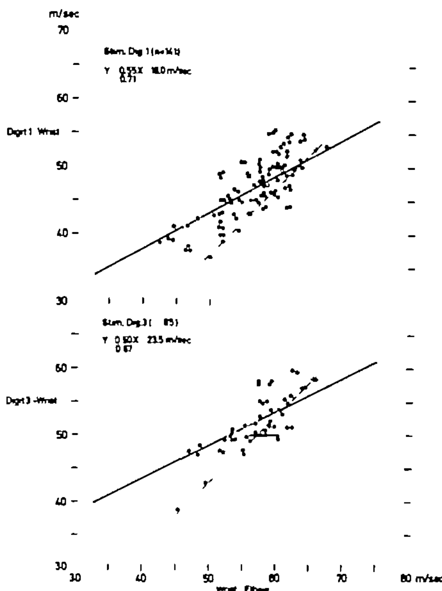


Fig 1 Sensory conduction velocity in the distal (*Y* axis) and proximal (*X*-axis) segments of the median nerve ●=CRF patients, O=patients after renal transplantation. The quadrangles illustrate the 95% of variation of the two variables in 48 normal subjects. Excluding measurements performed after renal transplantation the regression equations were: $Y = 0.49X + 17.9$ $r = 0.68$ (digit 1) and $Y = 0.47X + 24.2$, $r = 0.65$ (digit 3). --- $\Delta Y = \Delta X$

0.50 $r = 0.71$ and 0.67 $p < 0.001$ (digits 1 and 3 *respectively) The distribution of data around the* line $\Delta Y = \Delta X$ (stippled in Fig. 1) differed significantly from the normal ($p < 0.005$) indicating that the slowing of the conduction velocity was more pronounced in the proximal than in the distal segment. This was also true when expressed as the percentage deviation from the normal mean value which was higher in the proximal segment.

When the conduction velocities (stim digit 1 Fig. 2) were adjusted for the normal age variation (ΔV=m/sec) and related to the kidney function (log C_{Cr}) the intersection between the regression line and the lower 95% limit of normal variation (arrows in Fig. 2) corresponded to a creatinine clearance of 3 and 7 ml/min/1 73 m² in the distal *and proximal segments respectively This indi*cates that the slowing of the nerve conduction occurred earlier in the course of progressive kidney insufficiency in the proximal segment.

The same pattern was demonstrable in longitudinal studies in individual patients followed for months or years *until* renal transplantation or death. Fig. 3 shows the course in one patient who developed severe neuropathy In terminal renal failure the same action potential was conducted at a slower rate in the proximal than in the distal segment The ratio returned to normal several months after a successful renal transplantation For comparison Fig. 4 shows the

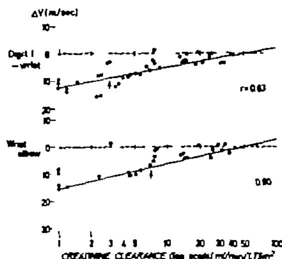


Fig. 2. Sensory conduction velocity in distal and proximal segments of the median nerve related to the kidney function (log scale) in patients who have not been subjected to regular dialysis treatment. The observation were expressed as the deviation (*ΔV m/sec*) from the age-adjusted mean value in normal subjects. Shaded area=95% range of normal variation (±2 S_{w} for the regression, conduction velocity on age) in 48 normal subjects. Arrows=the C_{Cr} level at which 50% of patient can be expected to show pathological conduction velocities. The regression equations were: *Y*=7.1*X*−12.5 *r*=0.63 (digit 1–wrist) and *Y*=9.1*X*−15.4 *r*=0.60 (wrist–elbow).

Fig. 3. Longitudinal course of the motor and sensory conduction velocity in the median nerve before and after transplantation (*R.A.T.* full vertical line) *H.D.* (stippled vertical line)=start of regular haemodialysis. White and black vertical bars to the left and right in the Figure=mean value ±2 S.D. in normal subjects. Note the intersection of the curves for distal and proximal sensory conduction velocity.

slowing of the sensory conduction velocity in proximal and distal segments recorded during 30 min of ischemia in a normal person. A similar course is apparent

DISCUSSION

These findings cannot be explained by differences in temperature proximally and distally. Furthermore they are incompatible with an ascending degenerative nerve lesion the commonly accepted *pathological picture in uremia (6). Due to the in*crease in the temporal dispersion of the compound action potential in uremia (10) the first positive peak to which the latencies were measured may not represent the same fibers at the wrist and elbow or from one examination to another. However by increasing temporal dispersion the first peak probably comes to represent slightly *faster* conducting fibers and more so the longer the conduction distance (4). Hence the different rate of slowing of the nerve conduction in distal and proximal segments may in fact have been underestimated. Studies in uremic patients comparable *to the present one are not available from the* literature. Kemble (7) reported that the index between the proximal and the distal conduction velocity was the same in normal subjects as in patients with uremic neuropathy. However he stimulated the nerve at two *different* levels and hence his data do not account for a possible slowing of the rate of impulse propagation in subsequent segments *along* the nerve.

The present results appear compatible with the hypothesis of a decremental nerve conduction reinstated and extensively discussed by Lorente de Nó and Condouris (8). This implies that the conduction velocity decreases as a function of the severity of the axon membrane dysfunction and of the length of the nerve segment through which the action potential is conducted. Both requirements seem fulfilled since as previously discussed (10) the impairment of the membrane function is related to the severity of the uremic intoxication in all probability resulting in a reduction of the resting membrane potential difference (3, 5). The uremic intoxication therefore may become included among the factors enumerated by Lorente de Nó and Condouris (8) which can lead to decremental nerve conduction.

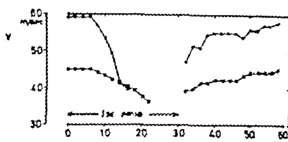


Fig. 4. The course of the sensory conduction velocity in the digit I–wrist (×) and the wrist–elbow (●) segment of the median nerve during and after 30 min of ischemia produced by inflation of a cuff placed around the upper arm to 200 mmHg. Normal subject, male 41 years old.

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RENAL FAILURE ASSOCIATED WITH GENTAMICIN THERAPY

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Abstract A report is given on 6 cases of impaired renal function possibly caused by treatment with gentamicin. All patients had normal renal function before initiation of treatment. Tw patients underwent peritoneal dialysis before recovery In all patients renal function returned to almost normal level within 150 days after withdrawal of gentamicin. The importance of an individualized dosage of gentamicin related to body weight and renal function as well as frequent control of renal parameters and antibiotic serum concentration during treatment is emphasized.

Gentamicin is the newest antibiotic of established clinical importance among the aminoglycosides, which also comprise streptomycin and kanamycin.

All aminoglycosides are known to be potentially nephrotoxic As regards gentamicin this was primarily demonstrated through animal experiments (1 5). The first experiences from the human clinic based on large patient materials showed a moderate impairment of the renal function in about % of the treated cases (3 11) Later reports have however described severe impairment of the renal function in connection with gentamicin therapy (9 13 22). In a nephrologic department 6 patients have within one year bee treated for nephropathy in which gentamicin must be presumed to occupy a central place in the etiology With a view to the extensive and increasing use of gentamicin publication of these cases is found justified

CASE HISTORIES

Case 1

A 5? year-old previously healthy man was admitted for Staph aureus bacteremia. Treatment was instituted ith penicillin and streptomycin after 6 day replaced by gentamicin, 80 mg three times daily methicillin, g four times daily and fusidic acid, 500 mg three times daily Serum creatinine and urography were at this time normal, but there was intermittent moderate proteinuria. The patient was at no time in shock

During gentamici therapy renal function became impaired and increasing proteinuria developed. After stopping of the treatment serum creatinine returned t normal level and proteinuria subsided. Vestibular examination showed almost discontinued estibular function, which was fully restored 1 month later (Fig. 1 Tables I and II).

Case 2

A 54-year-old previously healthy man w th Staph. aureus septicemia, which caused transient shock without any influence on the normal renal function. Treatment was instituted with penicillin and treptomycin, after day replaced by gentamicin, 80 mg three times daily and methicillin, g si times daily

During gentamicin therapy renal function became impaired and the patient developed oliguria and proteinuria. Microscopy of the urine sediment showed many erythrocytes, leucocytes, grained cylinders and erythrocyte cylinders.

Renal biopsy revealed pronounced tubulo-interstitial changes In places the tubular epithelium was necrotic in places in stat of regeneration and the tubular lumen contained prote and Hb cylinders, while the glomeruli were normal (sign. B Henriksen)

After tw dialyses the renal function had improved and serum creatinine returned to normal level. Gentamicin was still measurable in serum 4 days fter withdrawal. U rography performed before discharge was normal (Fig. 2, Tables I and II).

Case 3

A 72-year-old man developed ound nfection with Staph. aureus after operation for varices crurum, and treatment was instituted with gentamicin, 80 mg three times daily

During gentamicin therapy the renal function decreased, but after withdrawal of the drug serum creatinine returned to normal level The patient as at no time in shock. I rography before discharge was normal (Tables I and II)

Case 4

A 67-year-old man as admitted for cancer maxillae operation Due to complicating pneumonia, treatment was instituted with erythromycin, after 4 day replaced by gentamicin 80 mg three times daily On the last

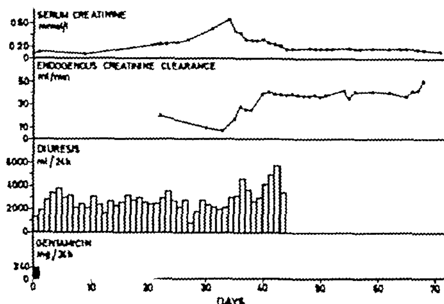


Fig 1 Case 1 Serum creatinine creatinine clearance diuresis and gentamicin dosage

days the treatment was supplemented by polymyxin 50 mg three times daily The renal function was normal at the beginning of the treatment, and the patient was at no time in shock.

During gentamicin therapy the renal function decreased and dialysis became necessary

About 3 month after withdrawal of gentamicin serum creatinine had returned to normal level. Three weeks after withdrawal, gentamicin was still measurable in serum. The serum concentration of polymyxin were all on a therapeutic level I urography before discharge was normal (Tables I and II)

5

A 75-year-old woman was admitted for treatment of colitis ulcerosa. The renal function was normal. Due to colon perforation with peritonitis laparotomy with ileostomy and colostomy was carried out Postoperatively the patient was treated with streptomycin and penicillin for 6 days then the treatment was changed to gentamicin 80 mg three times daily During the gentamicin therapy the renal function decreased, but after withdrawal of the drug it improved again Before the renal function was fully restored the patient developed a Candida albicans septicemia, and she died shortly after (Tables I and II)

Case 6

A 53-year-old woman with recidivating AV block was hospitalized for pacemaker implantation. The patient was cardially well compensated, and the renal function was normal Due to infection round the implanted pacemaker the patient was treated with gentamicin 80 mg three times daily in three period of 4 8 and 19 days, at intervals of 10 and 12 days respectively During the last period of treatment the renal function decreased, but after withdrawal of gentamicin serum creatinine slowly returned to normal level and the proteinuria

Table I *Changes in renal function during treatment with gentamicin*

	Serum creatinine (mmol/l) (normal ≤0 13)		Creatinine clearance (ml/min)		Proteinuria (g/24 h)		Duration of treatment (d)	
Case no	Before treatment	Maximum during treatment	Before treatment	Maximum during treatment	Before treatment	Maximum during treatment	Until renal function decreases	Total
1	0.09	0.68		7 9	0	4 0	14	22
	0.1	1 58		0 3	0 0	1 6	12	17
3	0.11	0.29		33 0	0 0		12	21
4	0 10	1.32		1 6	0.0		8	11
5	0.07	0.41		3 6	0.0	1 0	16	25
6	0 07	0.35	81 0	9 3	0 0	2.0	10	19

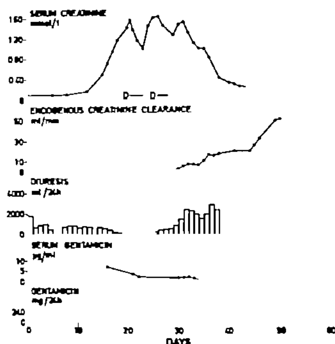


Fig. 2. Case 2. Serum creatinine, creatinine clearance, diuresis, serum gentamicin and gentamicin dosage. *D*=peritoneal dialysis.

stopped. I. v. urography before discharge was normal (Tables I and II).

Gentamicin was in all cases administered intramuscularly and the serum levels were measured immediately before an injection (predose level). All patients had normal liver function prior to and during treatment.

DISCUSSION

All the presented patients developed nephropathy in association with gentamicin therapy. It is, however, difficult to evaluate clinically the nephrotoxicity of gentamicin in these patients, who were often in a very poor condition and in whom factors like septicemia, hypotension or other medicamental treatment may have caused or contributed to the impaired renal function.

The following, however, supports the suspicion of gentamicin-induced renal damage: 1) In all patients the drop in renal function occurred some *time after the acute stage of disease including BP fall and septicemia.* 2) The renal function was normal at the beginning of gentamicin therapy and only started to decrease after 8–14 days of treatment. 3) The period of remission was extremely prolonged in all the patients as compared with uncomplicated shock kidneys, which are in most

Table II. *Dosage of gentamicin, duration of proteinuria and renal impairment*

Case no.	B.wt. (kg)	Gentamicin dosage (mg/kg/24 h)	Maximum serum predose level (µg/ml)	Oliguria	Duration of proteinuria after withdrawal of gentamicin (d.)	Duration of raised serum creatinine after withdrawal of gentamicin (d.)	Affection of vestibular organ
1	56	4.5	–	No	12	50	Yes
2	65	3.7	–	Yes	38	65	N
3	78	3.0	6.2	No	–	37	No
4	65	3.1	>10.0	Yes	365	90	N
5	45	5.0	>10.0	N	>14	>14	
6	53	4.5	1.5	N	27	150	No

cases more quickly restored 4) The renal biopsy revealed severe tubular damages but unaffected glomeruli. Similar histological renal changes have been demonstrated by animal toxicologic investigations with gentamicin (1 5 10 21)

However several of the patients have simultaneously with gentamicin received other potentially nephrotoxic drugs Thus cases 1 and 2 were treated with methicillin which is known occasionally to cause nephropathy probably through an allergic mechanism (17) Case 4 received polymyxin, which also belongs to the potentially nephrotoxic antibiotics (17)

Animal experiments have shown that dogs are particularly sensitive to the nephrotoxic properties of gentamicin as administration of -3 times the human therapeutic dose causes degenerative changes in the tubular epithelium and larger doses produce tubular necroses while glomeruli are unaffected (1)

After administration of large doses of gentamicin similar renal changes have been described in rats cats and monkeys (5 10 21) In all the experimental animals the cause of death was renal insufficiency and uremia

Animal experiments have proved that there is a certain although rough connection between the dose mg/kg/24 h and the frequency of nephropathy (1 5 21)

The pathogenesis of gentamicin nephropathy s to be merely toxic damage of the tubules as no justification for an allergic genesis has been found (4)

In the human medical clinic Falco et al (3) state the frequency of impaired renal function during gentamicin therapy to be about % judged from measurements of blood urea. The renal damages found were slight and reversible. No correlation was found to exist between total dose or the duration of the treatment and the frequency or degree of renal damage

Hewitt (9) has reported 3 cases of gentamicin-induced renal impairment and Kahn and Stein (13) have described a case of non-oliguric renal insufficiency in which the renal function was only partly restored 5 months after the end of the treatment.

Wilfert et al. (22) have reported gentamicin related reduction of renal function in 4 patients In all cases the renal function was fully restored

Opitz et al (16) refer to 10 cases of renal impairment in a group of 16 patients who were treated simultaneously with gentamicin and cephalothin All patients had normal renal function at the start of the treatment. Hewitt (9), Schultze et al (19) and Wilfert et al (22) have also described cases of renal insufficiency occurring during simultaneous treatment with gentamicin and cephalothin These and more recent cases (2, 4 15) imply that this combined treatment involves a special risk of renal impairment.

Summing up it must be admitted that there is no definite evidence of the pathogenetic importance of gentamicin in some of the patients covered by this study but based on the case records the histology of the renal biopsy and the reviewed animal experiments and clinical reports we find that this is the most probable cause in the cases described

By and large the observed cases of renal impairment were reversible serum creatinine being normalized in 5 patients after about 37–150 days (average about 80 days) The long periods of remission should be ascribed to the severe tubular damages. As no comparable clearance measurements were made before and after gentamicin therapy it is not possible definitely to evaluate the possibility of permanent reduction of renal function in the patients here concerned

Gentamicin is almost exclusively excreted renally by glomerular filtration (1 7). In 2 of the present patients gentamicin was still measurable in serum more than 3 weeks after the last administration This slow elimination may—besides the reduced renal function and the protein binding of gentamicin (1)—be ascribed to the fact that if the time of diffusion is sufficiently long, gentamicin is also to some extent capable of diffusing into the intracellular space (8) The diffusion back again of this intracellularly distributed gentamicin is undoubtedly a very slow process In peritoneal dialysis the elimination is insignificant (6 20). Therefore if reduction of the gentamicin content of the organism in patients with kidney insufficiency is indicated hemodialysis is the only efficient method (18)

The magnificent antibacterial properties of gentamicin have justified its extensive clinical use. The potential nephrotoxicity should however always be borne in mind The present case histories emphasize the risk of standardized treatment with gentamicin If oto- and nephrotoxic side-effects

are to be avoided. It is of the greatest importance to adapt and individualize the dosage not only according to body weight but also to renal function, which in ol es, before institution of the treatment, determination of serum creatinine, creatinine clearance, examination for proteinuria and microscopy of the urine sediment. Evaluation of the glomerular filtration exclusively based on serum creatinine is ot ad ocated. In the first place the correlation between serum creatinine and creatinine clearance is not linear and secondly the glomerular filtration may vary considerably according to age and sex without being reflected in serum creatinine (14). Guidance regarding individualized dosage in patients with reduced renal function is given by Gingell and Waterworth (7). During the treatment regular control of serum creatinine and creatinine clearance is necessary and at the least sign of decreasing renal function, which may reasonably be ascribed to gentamicin the treatment should be immediately discontinued. Determinations of serum concentrations are most helpful in the search for the optimal dosage and are considered necessary all through the treatment of patients whose renal function is already impaired.

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SERUM URIC ACID LEVELS IN WOMEN

An Epidemiological Survey with Special Reference to Women with High Serum Uric Acid Values

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Abstract Women in the age strata 38, 46, 50, 54 and 60, 1462 women in all, were submitted to a population study in Göteborg, Sweden. Determination of serum uric acid levels was included in the study. The mean values of serum uric acid increased from 3.6 mg/100 ml in women aged 38 to 4.4 mg/100 ml in women aged 60. The rise in serum uric acid with age seemed to be unrelated to the menopause as no differences were found between premenopausal and postmenopausal women of the same age. None of the 1462 women studied was found to suffer from clinical gout. When comparing women with high serum uric acid values and women in the general population of corresponding ages no significant differences were found for joint complaints, serum creatinine history of cystitis and/or pyelonephritis, history of renal calculi, alcohol consumption or socio-economic status. Women on saluretic diuretics had significantly raised serum uric acid values compared to the general population of women. The urine concentrating capacity was decreased in hyperuricaemic women both in those who took and in those who did not take saluretic diuretics. Women with high serum uric acid values had significantly higher values for body weight, subscapular skinfold and Hb. Serum uric acid was raised in women with arterial hypertension women with history suggesting angina pectoris and women with ECG changes suggesting ischaemic heart disease. However these differences were probably caused by an overrepresentation of women on saluretic diuretics in these groups.

Since the middle of the 18th century it has been known that there is an association between high uric acid levels and clinical gout (27) However as found from previous population studies, all people with high serum uric acid levels do not develop clinical gout (16, 21). A possible association between high serum uric acid values and ischaemic heart disease has also been discussed though previous population studies do not definitely support such an association (15 22) An association has been found between high arterial BP and high serum uric acid levels (9 17) Furthermore saluretic diuretics are known to raise serum uric acid values (10)

The present paper presents further data on these matters. Serum uric acid levels in a population sample of women are presented Some characteristics of women with high serum uric acid values are given. Three groups of women participating in the population study are discussed separately- those on saluretic diuretics those with high BP who are not on saluretic diuretics and those with symptoms or ECG changes suggestive of ischaemic heart disease

MATERIAL AND METHODS

A population study of women in Göteborg, Sweden, was performed in 1968–69 (7) Women in the age strata 38 46, 50 54 and 60 were examined (Table I), altogether 1462 women (participation rate 90.1%) Women born on dates which were even multiples of six were called for the study (7). I this way the population sample was considered to be representative of the female population in Göteborg in the age strata studied. The survey was performed for the most part during 12-month period. In this way the influences of age differences within each age group and of seasonal variation were reduced as far as possible

Blood sampling was performed in the morning after overnight fasting (7) Serum uric acid level were determined manually by an enzymatic method essentially according to Praetorius (26) using Uricase L (Levens Kemiske Fabrik, Denmark) on deep-frozen sera (−20°C). The day-to-day variation (S.D × 100/mean) was found to be 4.1% Values within the upper five centiles of

Table I *Serum uric acid levels (mg/100 ml) in a population sample of women in Göteborg 1968–69*

Age (y)	n	Mean value	S.D	S.E	Median value	Range
38	368ᵃ	3.6	1	0.06	3.5	0.9– 9.0
46	479ᵇ	3.8	1.1	0.05	3.7	0. – 8.0
50	396ᵇ	4.1	1.2	0.06	3.9	1.0–11.6
54	176ᵃ	4.3	1.4	0.11	4.0	1.6–10.9
60	81	4.4	1.5	0.17	4.2	0.6– 9.0

4 data missing. data missing.

the population sample in the different age strata were defined as high values (hyperuricaemia)

Twenty-nine women in the population sample reported history of angina pectoris (6) as defined according to Rose (28) ECG changes implicating Minnesota Codes 1 1–, 4 1 5 1 2 (in the absence of 3 1) 6 1 7 1 (3 8) were defined as coronary ECG and recorded in 23 women participating in the population study (6)

Statistical methods Conventional methods were used for calculation of median value mean value standard deviation (S.D) and standard error of the mean (S E.) The hypothesis of differences in frequencies between groups was tested by means of the binomial distribution with a normal approximation (6) hypothesizing that the characteristics studied were more common in women with high serum uric acid values (one-tailed test). Significance of differences between mean values was estimated using Student *t*-test.

RESULTS

Serum uric acid levels in the female population

Table I shows mean values S D S E median values and ranges of serum uric acid values in the different age strata studied There is a rise with age from 3.6 mg/100 ml in women aged 38 to 4.4 mg/100 ml in women aged 60 Fig 1 shows the distributions of serum uric acid values The distribution is normal in women aged 38 except for a few extremely high values while the distributions of women aged 46–60 are somewhat skewed to the right with mean values higher than median values

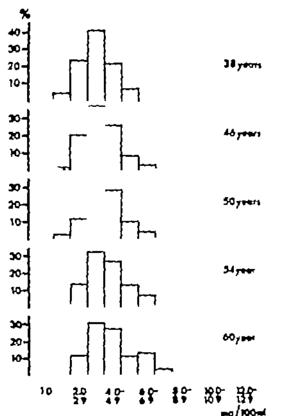


Fig 1 Distributions of serum uric acid values in a population sample of women in Göteborg 1968–69

Serum uric acid levels were found to rise with age also when women of similar body weight in the various age strata were compared (Table II)

Comparison between pre- and postmenopausal women In Table III the women participating in the population study are subdivided into pre- and postmenopausal women No differences were found in serum uric acid levels between pre- and postmenopausal women of the same age Women with surgical menopause are not included in the Table as it could often not be established whether

Table II *Serum uric acid levels (mg/100 ml) in women of similar body weight (kg)*

Age (y)	B wt. 55 0–59 9 n	Mean	S D	B wt. 60 0–64 9 n	Mean	S D	B wt. 65 0–69 9 n	Mean	S D
38	81	3 2	0 9	76	3.5	1 1	49	3 7	1 0
46	96	3 7	1 1	114	3 6	1 0	62	3.8	0.9
50	66	3.9	1 3	90	4 0	1 2	77	4 1	1 0
54	25	3 7	1 3	39	4 3	1 1	79	4.5	1 3
60	13	3.8	0.5	15	4 2	1.6	16	4 8	1.5

Table III. *Serum uric acid levels (mg/100 ml) in pre- and postmenopausal women in the population sample*

Age (y)	Premenopausal			Postmenopausal[a]			Signif cance of difference
		Mean	S.D		Mean	S.D	
38	347	3.6	1.2		–	–	
46	347	3.8	1 1	33	3 9	1 0	N.S
50	17?	4 1	1	146	4 0	1 4	N.S
54	10	4.5	1 0	138	4.2	1.3	N.S
60	–		–	71	4 4	1 6	

[a] No menstruation during the last 6 months; women with surgical menopause not included

Table V *Borderline values (mg/100 ml) for hyperuricaemic women*

Age (y)	Borderline upper 5 centiles
38	5.5
46	5.8
50	6.1
54	6.7
60	7._

both ovaries had been removed in women who had been subjected to hysterectomy. There was no correlation between serum uric acid levels and time since last menstruation either as will be seen from Table IV which shows serum uric acid values in postmenopausal women according to time since last menstruation.

Characteristic of women with high serum uric acid values

Women with serum uric acid values within the upper five centiles in the different age strata (Table V) have been defined as hyperuricaemic. Some characteristics of them (different age strata taken together) are shown in Table VI.

Clinical gout None of the participants in the population study had a history of clinical gout.

Joint complaints The participants were asked for finger joint complaints. Finger joint complaints were not more common in hyperuricaemic women than in women in the population sample irrespective of whether a history of or the prevalence of finger joint complaints at the time of the population study were taken into consideration.

Arterial hypertension and use of saluretic diuretics Serum uric acid in hypertensive and non-hypertensive women and in women who took and those who did not take saluretic diuretics will be dealt with below. As may be seen from Table VI, a history of arterial hypertension was about 3 times as common and the use of saluretic diuretics about 4 times as common in the hyperuricaemic women as in the total population sample.

Kidney diseases Proteinuria was significantly more common in hyperuricaemic women. The difference disappeared if women on saluretic diuretics were excluded. Thus 37% of the hyperuricaemic women who took saluretic diuretics had proteinuria compared to 7% of those who did not take and to 6% of women in the total population sample.

The urine concentrating capacity was decreased in hyperuricaemic women. A decrease (<600 mOsm/kg H_2O after 13 hours thirst) was noted in 36% of hyperuricaemic women who took saluretic diuretics but there was also a difference between hyperuricaemic women who did not take saluretic diuretics (22%) and the women in the total population sample (1 % $p<0.05$)

A history of renal calculi (doctor's diagnosis) was as common in the total population sample as in the hyperuricaemic women. There were no sig-

Table IV *Serum uric acid levels (mg/100 ml) in postmenopausal women according to time since last menstruation*

Age (y)	Time since last menstruation: 2–11 mo			1–4 y			5–9 y			≥10 y		
		Mean	S D		Mean	S D		Mean	S D		Mean	S D
46	28	3 7	1 0	33	3 6	1 1	12	4.2	0 9	5	3 6	0 6
50	51	4._	1 4	116	4 0	1.3	32	3 9	0.8	16	3 8	1 1
54	17	3 9	1 4	60	4.2	1.3	62	4 4	1 7	26	4 3	1 1
60		–	–				18	4 4	1 9	60	4 4	1 4

Table VI *Some characteristics of hyperuricaemic women compared to the total population sample*

Variable studied	Hyperuricaemic women (%)	Total population sample (%)	Significance of difference
Clinical gout	0	0	N.S
History of finger joint complaints	16 0	14.5	N.S
Prevalence of finger joint complaints causing a visit to a doctor	2.7	6.2	N.S.
History of arterial hypertension[a]	24 0	8.6	$p<0.001$
Use of saluretic diuretics	21.3	5 0	$p<0.001$
Proteinuria	13 7	6.3	$p<0.01$
Urine concentrating capacity <600 mOsm/kg H_2O[b]	25 0	11.8	$p<0.001$
History of renal calculi	5.3	5.3	N.S
History of cystitis and/or pyelonephritis	38 7	40.8	N.S
History of pyelonephritis	9.3	6.5	N.S
Beer several times a week	28.4	26.5	N.S.
Wine several times a week	2.7	4 9	N.S
Spirits several times a week	0	1.2	N.S
High socio-economic status[c]	1 4	6.0	N.S
High socio-economic status of husband	13.6	13.8	N.S.

During pregnancy only not included.
After 13 hours thirst.
E.g. large scale employers and officials of high or intermediate rank (6).

nificant differences concerning history of cystitis and/or pyelonephritis.

Alcohol consumption No significant differences were found between hyperuricaemic women and women in the total population sample when comparing the numbers of women who consumed alcohol several times a week

ocio-economic status There were no significant differences in socio-economic status. If anything, the socio-economic status tended to be lower in hyperuricaemic women. No differences were found in socio-economic status concerning the husbands of the women either

Anthropometric data Table VII shows some anthropometric data on hyperuricaemic women compared to the total population sample in the different age strata. There were no significant differences in body length Body weight tended to be higher in hyperuricaemic women in all the age strata studied. Significant differences were found for women aged 38 and 54 Similarly the subscapular skinfold was thicker in the hyperuricaemic women. Significant differences were found for women aged 38 46 54 and 60

Haemoglobin The Hb values were found to be higher in the hyperuricaemic women than in the total population sample in the different age strata studied. Significant differences were found for women aged 38 46 and 54 (Table VII)

Serum uric acid in women on saluretic diuretics

As shown in Table VI hyperuricaemic women were significantly more often on saluretic diuretics than the women in the total population sample. As seen from Table VIII participants on saluretic diuretics also had significantly higher serum uric acid levels than the total population sample in the different age strata studied

Serum uric acid in women with arterial hypertension

Table IX shows serum uric acid levels in women who received saluretic diuretics because of arterial hypertension at the time of the population study and of women who had hypertension (systolic BP ≥160 mmHg in sitting position) but who were not on saluretic diuretics at the time of the study Women who took saluretic diuretics because of hypertension had significantly higher serum uric acid values in all the age strata studied while the values were similar in women with systolic BP ≥160 mmHg who were not on saluretic diuretics and in the total population sample in the age strata studied

Serum uric acid in women with angina pectoris and coronary ECG

As will be seen from Table X women in the population sample who had angina pectoris ac-

Table VII *Anthropometric data and Hb levels of hyperuricaemic women compared to the total population sample*

Age (y.)	Hyperuricaemic women			Total population sample			Significance of difference
		Mean	S D		Mean	S D	
Body length (cm)							
38	19	164.3	5 9	372	164.6	5 7	N.S
46	22	162.3	5 8	431	163.5	7.5	N.S
50	20	163 4	4.8	398	163.5	5.5	N.S
54	9	166.1	9 7	180	162 6	5 4	N.S
60	5	160.6	6 4	81	162 0	5 1	N.S.
Body weight (kg)							
38	19	74 1	22.3	372	63 4	11.[illegible]	$p<0.001$
46	22	66.1	12.1	431	63 0	9.8	N S.
50	20	69.2	11.9	398	66.2	11 1	N.S
54	9	81 1	17 1	180	66.0	12.5	$p<0.001$
60	5	68 4	11.8	81	66 1	10 9	N.S
Subscapular skinfold (mm)							
38	19	22.5	10.5	372	15.3	7.[illegible]	$p<0.001$
46	22	19.3	8.1	430	16.1	7 1	$p<0.05$
50	20	19.3	8.3	398	18.8	8.2	N.S.
54	9	26.3	10 8	180	18.4	8.2	$p<0.01$
60	5	25 6	9 1	81	19 6	8.5	$p<0.01$
Hb (g/100 ml)							
38	19	14 0	0.97	372	13.5	1.02	$p<0.05$
46	22	14 0	1 00	431	13.5	1 15	$p<0.05$
50	20	13.8	1.27	398	13 7	1 10	N.S.
54	9	14 7	1 02	180	13.8	0 99	$p<0.01$
60	5	14.5	0.77	81	13.8	0.85	N S

cording to the definition used (mean age 51 3) had significantly higher serum uric acid values than a reference group of women of similar mean age (the participants aged 50) However when the women with angina pectoris were subdivided into those who took and those who did not take saluretic diuretics, the difference was found to be caused by those on saluretic diuretics only Thus the mean value was found to be 4.2 mg/100 ml in the women with angina pectoris who did not take saluretic diuretics compared to 4 1 mg/100 ml in the reference group As shown in Table X women with "coronary ECG (mean age 54 2) had significantly higher serum uric acid levels than a reference group of similar mean age (the participants aged 54). However this difference could also be shown to be caused by those in the coronary ECG" group who took saluretic diuretics. If these women are excluded the mean value was found to be 4.3 mg/100 ml both in the group of women with coronary ECG and in the reference group

Table VIII. *Serum uric acid values (mg/100 ml) of women on saluretic diuretics in the population sample and of the women in the total population sample*

Age (y)	On saluretic diuretics			Total population sample			Significance of difference
		Mean	S.D		Mean	S D	
38	6	5.2	1 4	368	3.6	1	$p<0.01$
46	11	4.6	1.3	429	3.8	1 1	$p<0.05$
50	19	5 0	1 6	396	4 1	1 2	$p<0.001$
54	21	5.6	2.0	176	4.3	1 4	$p<0.001$
60	15	6.1	1 5	81	4 4	1.5	$p<0.001$

Table IX. *Serum uric acid values (mg/100 ml) of hypertensive women on saluretic diuretics in the population sample of women with systolic BP ≥160 mmHg at the population study examination who were not on saluretic diuretics and of the women in the total population sample*

Age (y)	Hypertensives on saluretic diuretics			Systolic BP ≥160 not on saluretics			Total population sample		
	n	Mean	S.D	*n*	Mean	S.D	*n*	Mean	S.D
38	3	5.3	2.1	3	4.7**	0.9	368	3.6	1.2
46	10	4.6	1.4	22	3.7*	1.0	429	3.8	1.1
50	15	4.8	1.5	46	4.3*	1.6	396	4.1	1.2
54	18	5.9*	2.0	[illegible]	4.4*	1.2	176	4.3	1.4
60	10	5.9*	1.5	24	4.0*	1.4	81	4.4	1.5

$p<0.05$ $p<0.01$ *** $p<0.001$ significantly different from women in the general population.
Not significant.

DISCUSSION

The results presented in this paper derive from a population study of a representative sample of women in five age strata in a defined urban area. Due to the means of selection and the high participation rate the results are considered to be valid for the population studied.

The serum uric acid levels were found to rise with age in the present study which finding agrees with previous results elsewhere as reviewed by Neel (23) and as will be seen in Fig. 2 in which the present results are compared with those from some previous studies of women. The Göteborg values seem to have an intermediate position and agree most closely with those reported from Framingham, USA (16) Saloinen Finland (17) and Wensleydale UK (25) As determinations may be made on fresh or deep-frozen sera and different methods for determination of serum uric acid are used owing to which some differences may be expected (11 23 24) It seems unwise to conclude that serum uric acid levels are definitely different in women in different parts of the world

Previous studies indicate that serum uric acid levels are stable in males from puberty onwards and about 1 mg/100 ml higher in men than in women until the time of the menopause of the women (23) This also agrees with the results of the present study and a previous population study of men in Göteborg. Mean serum uric acid level was found to be 5.3 mg/100 ml in 50-year-old men (32) compared to 4 1 mg/100 ml in 50-year-old women

The rather constant finding in previous studies of a fairly steep rise in serum uric acid during the climacteric age but rather stable levels before this age (16 17 1 24 25 30) has led to the assumption that the rise in serum uric acid during the 5th and 6th decades is caused by the menopause However there seems to be no previous study in which pre and postmenopausal women of the same age are compared. In the present study

Table X. *Serum uric acid values (mg/100 ml) of participants in the population study with angina pectoris and coronary ECG and in the total number of participants in the age strata 50 or 54*

	Whole group			On saluretics			Not on saluretics			Whole group		
	n	Mean	S.D	*n*	Mean	S.D	*n*	Mean	S.D	*n*	Mean	S.D
Angina pectoris / *Population sample aged 50*	29	4.9*	1.7	7	7.0*	1.3	22	4.2*	1.1	396	4.1	1.2
Coronary ECG / *Population sample aged 54*	23	5.0*	1.6	12	5.6	1.5	11	4.3*	1.3	176	4.3	1.4

$p<0.05$ $p<0.01$ *** $p<0.001$ significantly different from the women in the general population
Not significant.

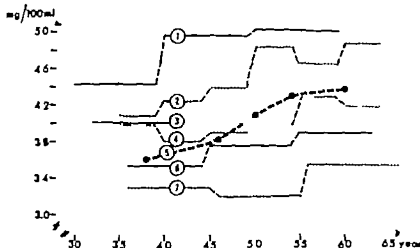


Fig. 2. Means of serum uric acid levels in women in the present study and in some previous surveys. 1=New Zealand (12), 2=Tecumseh, USA (21), 3=Framingham, USA (16), 4=Finland (17), 5=the present study, 6=Wensleydale, UK (25), 7=DDR (30).

age strata instead of age groups were used. In this way pre- and postmenopausal women of almost identical age could be compared and no differences were found between them. The rise in serum uric acid levels with age in women thus seems to be unrelated to the menopause.

The prevalence of clinical gout is lower in women than in men (16, 18, 24, 29). All hyperuricaemic subjects do not suffer from gout. Thus 3 of 12 women in Framingham who had serum uric acid values ≥7 mg/100 ml at a single examination had had clinical gout at the 12 year follow-up (16). In the present study none of the women was known to suffer from clinical gout. In view of previous studies of women this could be anticipated. In two studies in Japan none of about 14000 women was found to have gout (18, 29). There was no overrepresentation of women with joint complaints among those with high serum uric acid values in the present study either.

Saluretic diuretics are known to raise serum uric acid levels (10, 17, 31). A significant difference between those who took and those who did not take saluretic diuretics was also found in the present study. The differences were found to be about 1.0–1.5 mg/100 ml in the different age strata. These figures also agree with those found in a subsequent study of hypertensive participants (5) comparing placebo, chlorthalidone (a saluretic substance) and alprenolol (a β-receptor blocking agent). Serum uric acid levels were on an average 1.4 mg/100 ml higher on chlorthalidone than on placebo.

There seems to be an association between high serum uric acid levels and kidney disease (16, 17) and between gout and renal stones (24, 33). In the present study no differences in serum creatinine levels or in history of cystitis and/or pyelonephritis were found between women with high serum uric acid values and the total population sample, nor was there any difference regarding history of renal calculi. There were very few women with badly impaired function as judged from serum creatinine values in the population sample. The results therefore do not exclude an association between high serum uric acid and kidney disease which seems to be present in subjects with severely impaired renal function (14).

Proteinuria was significantly more common in women with high serum uric acid values in the present study than in the total population sample. This disagrees with the results from Framingham where no significant difference was found between hyperuricaemic subjects and those in the total population sample (16). However, the difference in the present series seems to be caused by overrepresentation of women on saluretic diuretics among those found to be hyperuricaemic and overrepresentation of women with proteinuria among those taking saluretic diuretics. When women on saluretic diuretics are excluded, no difference is found between hyperuricaemic women and women in the total population sample, which is then in agreement with the results from Framingham, as the serum uric acid determinations in Framingham were carried out before the introduction of saluretic diuretics.

Tubular function measured as urine concentrating capacity was decreased in hyperuricaemic women in the present study. This difference is

obvious even when women on saluretic diuretics are excluded It seems possible that tubular damage may cause hyperuricaemia or that hyper uricaemia may cause tubular damage There seem to be no other reports from population studies on the association between serum uric acid and urine concentrating capacity

Some investigators have noted an association between arterial hypertension and high serum uric acid levels (9 17 19 30) while others have found no or at most a very weak such association (1? 15 22) The study of Isomäki (17) though carried *out on hospitalized subjects is of especial interest*, as hypertensive subjects who were and who were not on saluretic diuretics were also studied separately The differences in serum uric acid between hypertensive subjects and subjects in the general population were reduced when those on saluretic diuretics were excluded but the author concluded that saluretic diuretics could not explain all the difference In the present study women with high serum uric acid values had significantly more often a history of arterial hypertension and hypertensive women had significantly higher serum uric acid values than women in the general population. However this difference seemed to derive entirely from those hypertensives who took saluretic di uretics, as no differences were found when they were excluded.

A possible association between ischaemic heart disease and serum uric acid has been discussed (4). No association was found in Tecumseh USA (22). In Framingham, USA an association was found between gout and ischaemic heart disease but not between high serum uric acid and is chaemic heart disease (15) Isomäki (17) reported from Finland an association between high serum uric acid and ischaemic heart disease (hospitalized subjects) which mostly disappeared when women on saluretic diuretics and women with high serum creatinine values were excluded In the present study an association between ischaemic heart dis ease and high serum uric acid values was found both when studying women with a history suggesting angina pectoris and women with ECG changes suggesting ischaemic heart disease How ever many of the women classified in these two categories were on saluretic diuretics, and if these women were excluded no differences remained

An association between serum uric acid and body weight has consistently been reported in previous studies (2, 12 16 19 24 30) An association has also been reported between skinfold and serum uric acid (12) and between body weight and gout (16 24). In the present study body length was similar in women with high serum uric acid and in the general population, while the values for body weight and subscapular skinfold were higher in the women with high serum uric acid The differences were mostly statistically significant. They were not attributable to saluretic diuretics as women with high body weight and women in the general population did not differ in this respect.

The difference in subscapular skinfold may indicate that differences in body fat are at least partly responsible for the association between body weight and serum uric acid

Like serum uric acid body weight increased with age (Table VII) The rise in serum uric acid with age might thus be secondary to the rise in body weight with age and non-related to age per se In order further to elucidate this question, women of similar body weight in the different age strata were compared as to serum uric acid values. *As shown in Table II serum uric acid levels rose* with age in women of similar body weight Thus at least part of the rise with age in serum uric acid levels was non-related to body weight and the rise with body weight non-related to age.

An association between Hb and serum uric acid has been reported in males (2, 1) One study noted an association in women also (2) while another failed to reveal such an association in women (12) Significant differences between women with high serum uric acid and the general population of women were found in three of five age strata studied in the present series

One reason may be a higher rate of cell destruction in healthy subjects with a large red cell volume, which may be the case in polycythaemia and leucaemia (13). The difference in red cell mass between men and women (6) might possibly explain some of the sex difference in serum uric acid levels. Lower serum uric acid levels in women of premenopausal than of postmenopausal age and in men have been considered to be explained by the loss of cells occurring in the menstrual flow of premenopausal women (2) However this is not in accordance with our findings, which did not indicate any difference in serum uric acid levels between premenopausal and postmenopausal women of similar age.

According to previous studies there is an association between high alcohol consumption and high serum uric acid values in men (1 17 31) There seems to be no study which has revealed such an association in women nor did the present one do so The reason is probably the low alcohol *consumption of women compared to men (6)* and the rapid decrease in serum uric acid after the last intake of alcohol (20).

According to medical tradition it is said that gout is a disease of wealthy people However previous studies indicate that high serum uric acid levels are more common in subjects of low socio-economic status (1) and a similar tendency was noted in the present study although the difference was not statistically significant

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PROGNOSIS IN SEVERE CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Abstract The prognosis of 187 patients with severe chronic obstructive pulmonary disease has been evaluated by use of the life table method. All patient had FEV less than 60% of VC and below 1.5 l. The observation time ranged from 4 years and 4 months to 7 years and 4 months. The cumulati survival rate in the whole material was 71% after 3 years and 47% after 6 years, results which are very close to the survival found by other authors. Calculations are presented of the survival of FEV subgroups and of cli ical functional groups separately and combined. These two classifications have a good correlation to survival, and it is demonstrated that the combination of functional group and FEV subgroups adds more information regarding the prognosis than that obtained through FEV alone. Among patients with earlier pulmonary tuberculosis the prognosis was related only to the degree of pulmonary obstruction. The expected increase in lethality with advancing age was demonstrated, but the mortality was relatively higher in the younger age group when compared to the general population of the same age. The prognosis of patients with cor pulmonale was considerably worse than of patients without cor pulmonale even when the higher degree of severe airway obstruction was considered.

The prognosis of chronic obstructive pulmonary disease has been shown to be related to several features of the disease, such as vital capacity (VC) (3 4 5 22), maximal breathing capacity (1 3 4, 16), forced expiratory volume in first second (FEV) or mid-expiratory flow rate (5 14 20 22 24), residual volume pulmonary diffusing capacity for CO (1 ., 3 5 10 20) radiological evidence of emphysema (23) cardiac disease especially cor pulmonale (3 5 10 14 20 22 23) clinical functional group (5 13 17 19), arterial oxygen saturation and CO_2 tension (4 10 14 20 23 25) polycythaemia (19) early age at onset of disease or symptoms (16 18 1 23) residence at high altitude (22) and weight loss (3 4 26)

Several of these features are interrelated and associated with expiratory slowing (5 26) and when their relative value for prognosis was examined (5), it turned out that if FEV was the same then the prediction of survival was improved mainly by resting heart rate (higher heart rate lower survival) overall assessment of functional class, history of edema and to a smaller extent by ECG signs of cor pulmonale cl nical evaluation of functional test improvement in forced expiratory olume with isoproterenol and by albumin/globulin ratio It seems also that the prediction of the prognosis is improved by consideration of the decrease in FEV over a year period (5). The prognosis in relation to FEV seems n different studies to be nearly the same and patients with a FEV above 1.5 l have a prognosis of survival not much different from a normal population of the same age and sex (5 6 22). *The FEV is the parameter best correlated* to the clinical functional evaluation (7 8 9)

The present report describes the prognosis of survival for patients with severe chronic obstructive pulmonary disease n relation to the value of FEV and to the clinical functional group separately and combined

MATERIAL AND METHODS

Patients from Medical Departments B C and P Bispebjerg Hospital, Copenhagen, were incorporated in this study if they had had pulmonary function test performed in the period Jan 1st 1964 – Dec 31st 1966. Included for further study were patients who had a FEV below 1.5 l if this was les than 60% of VC Patients with pulmonary carcinoma or other cancers present at the time of the pulmonary functional test were excluded

Evaluation of clinical functional group was made according to the cla sification used by Jensen et al (13). Although it might have been desirable to use more detailed lassification, this was not possible with the information obtainable from the patient files.

Table I *Age and sex distribution FEV₁ and clinical functional group of the patients*

No. of pats.	Age (y)	Sex	FEV (l/sec) 0.0–0.6	0 7–0.9	1 0–1.2	1 3–1.5	Functional group A	B	C	Tub pulm.
1	0–29	♂	0	1	0	0	1	0	0	0
0		♀	0	0	0	0	0	0	0	
2	30–39	♂	0	1	0	1	1	1	0	4
6		♀	1	2	1	2	2	3	1	
20	40–49	♂	2	7	7	4	4	15	1	18
13		♀	1	5	7	0	5	6	2	
47	50–59	♂	9	15	16	7	6	31	9	29
23		♀	4	8	9	2	3	18	1	
56	60–69	♂	6	27	15	8	11	33	11	20
11		♀	6	4	0	1	2	4	1	
7	70–79	♂	2	5	0	0	0	2	5	0
1		♀	0	0	1	0	0	0	0	

The study was terminated on April 1st 1971 The longest observation period was 7 years and 4 months and the shortest 4 years and 4 months. To get the maximum information, the life table method was used for calculation of the cumulative survival rate (survival)

The standard errors of the 1st, 3rd and 5th years accumulated survival rates are worked out from the tables of Ederer (11) which are computed from an approximation to Greenwood's formula. Standard error values based on less than 50 cases are not shown, because rates based on less than 50 cases are too unreliable to be of value The fate of all patients by the 1 of April 1971 wa known and the date of death also known for all patients dying during the observation period

RESULTS

One hundred and eighty-seven patients, of whom 54 were women were selected in the way described Table I shows the age and sex distribution in relation to FEV and clinical functional group The mean age for the whole material is 57 years (range 20–79) and the mean FEV 0 9 1 S D 0.25 l (range 0.35–1.50)

The survival in the whole material was 71% after 3 years 63 after 4 57 after 5 and 47 after 6 years as seen in Table II The survival for subgroups according to FEV is seen in Fig. 1 The 6-year survival of patients with an initial value of FEV below 0 6 l is 22% 37% for those with FEV₁ 0 7–0.9 1 53% for those with FEV 1.0–1.2 l and 78% for those with FEV 1 3–1.5 l In a normal population" from Copenhagen of the same age and sex as the 187 patients 87% survived for 6 years (Statistical Yearbook 1967)

Among men below 60 46% were dead after 6 years of observation this percentage is more than 6 times as high as would be expected in a "normal population of the same age in Copenhagen. Out of 63 men above 60 years, 66% died during 6 years of observation. This is 2.2 times more than expected (Table III)

Patients with earlier active tuberculosis were included in the study if the lung function test was as described After 6 years 60% of the patients with earlier active tuberculosis of the lungs are still alive When it is considered that the FEV values are better in this group than in the whole material, there is no difference regarding prognosis of survival.

The clinical functional group could be estimated in 179 patients In the best of 3 groups group A

Table II *Survival of 187 patients followed to the end of the study in April 1971*

Follow-up (y)	At risk	Dying	Lost*	Cumulative proportion surviving %	S E.
0–1	187	23	0	88	4
1–2	164	16	0	79	
2 3	148	15	0	71	7
3–4	133	14	0	63	
4–5	119	10	27	57	9
5–6	82	11	31	47	
6–7	40	2	25	44	
7–8	13	0	13	44	

* Patients observed for fewer years

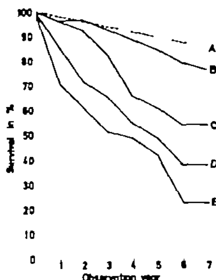


Fig. 1. Cumulative survival rate (life table method) of 187 patients according to FEV subgroups. *A* = survival of "normal population" of same age and sex. *B* = 25 patients with FEV 1.3–1.5 l. *C* = 56 patients with FEV 1.0–1.2 l. *D* = 75 patients with FEV 0.7–0.9 l. *E* = 31 patients with FEV <0.6 l.

(patients fit for light work), there was a survival of 76% after 6 years. In group B (patients able to climb staircases but with difficulty or able to walk short distances on level ground but unable to do more than the lightest work) 48% survived after 6 years, and in group C (patients confined to house or bed because of respiratory distress, unable to do even light work) the survival after 6 years was 15% (Fig. 2). The relation between clinical functional group and FEV is seen in Table IV and seems to be reasonably good.

The clinical functional group B is quite large and when it is subdivided into two groups according to FEV below and above 0.9 l, there is

Table III. *Survival of men according to age*

	≥60 y (*n* = 63)	<60 y (*n* = 70)
FEV ≤0.9 l	40	35
FEV 1.0–1.5 l	23	35
% dead by end of 6th y	66	46
% expected dead in "normal population" of same age after 6 y	29	7.1
% increase in death rat: found/expected	2.2	6.8

Table IV. *Relation between clinical functional groups A, B and C and FEV groups*

	Functional group			
FEV (l/sec)	A	B	C	Non-evaluable
–0.6	1	15	12	3
0.7–0.9	10	47	17	1
1.0–1.2	14	36		4
1.3–1.5	10	15	0	0

a 6 years' survival of 35% and 63% respectively (Fig. 3).

Patients with ECG signs of cor pulmonale, i.e. right ventricular hypertrophy or P waves more than 3 mm, were in an overall worse functional group and had a lower FEV value than the rest of the patients (Table V). The patients with cor pulmonale showed a 31% survival after 6 years, whereas 55% of those without cor pulmonale were alive after 6 years (Fig. 4).

If a calculation is made according to FEV values for patients with and without cor pulmonale, it is found that among the cor pulmonale patients there should have been 51% deaths in 5 years but 65% occurred, and among patients without cor pulmonale one would have expected 43% but only 36% occurred. These differences are not statistically significant within the 0.05 confidence level, but highly suggestive.

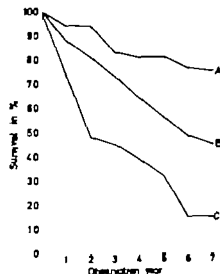


Fig. 2. Cumulative survival rate (life table method) of 179 patients according to clinical functional groups. *A* 35, *B* 113 and *C* 31 patients.

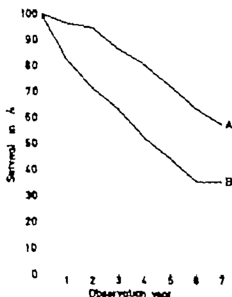


Fig 3 Cumulative survival rate among patient in clinical functional group B with FEV above and below 0.9 l A = FEV 1 0–1 5 l. B = FEV ≤0.9 l.

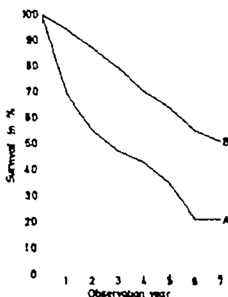


Fig 4 Cumulative survival rate among patients with (A) and without cor pulmonale (B).

In order to facilitate a comparison with other materials the 3 years cumulative death rate for all groups the S D and the 0.05 confidence level are presented in Table VI

DISCUSSION

The patients investigated include more women than most other similar materials There is no definite explanation for this but probably it only reflects different methods of selection and it must be *realized that all such materials are highly* selected It is unlikely that chronic obstructive pulmonary disease is more common among women in Denmark than in other countries In prevalence studies among an urban Swedish population (12 15) it was found that the relation between men and women with chronic bronchitis was 2 7/1.5–2.1/1.8 depending on the age groups studied The prognosis of survival after 3 and 5 years according to FEV_1 is very close to the survival found in the studies by Burrows and Earle (5 6) and Renzetti et al (22). These authors examined patients of comparable ages and with the same FEV values

The clinical functional group has a quite good correlation to the FEV demonstrated and the prognosis based on functional group alone shows a significant difference between the groups after 3 as well as after 6 years of observation If the functional group and FEV are combined there is an increase in selectivity regarding groups with a better and a worse prognosis The results for the smaller groups A and C have not been shown in combination with FEV because the S.D with so small a number of patients will not allow evaluation with any degree of significance But the tendency is the same and it seems that clinical functional group adds information regarding prognosis to the information obtained through FEV alone.

The results for patients with earlier pulmonary *tuberculosis showed no significant difference* from results among patients without earlier known tuberculosis but with a similar degree of pulmonary obstruction. These results are in good accordance with the report of Martin et al. (17) who in 611 patients with earlier tuberculosis found mortality to be related to the degree of airway obstruction. They also found that when the tuberculosis was treated the prognosis was related to the remaining degree of pulmonary obstruction

Age has a significant influence on survival. Men above 60 years had a lethality of 66% after 6 years of observation, while men below 60 years had a mortality of 48% This difference might, however be due to the higher number of men with a severe airway obstruction in the older group than in the younger But the relative increase in

Table V Patients with ECG signs of cor pulmonale in relation to age FEV and clinical functional groups

	Cor pulmonale	
	Positive	Negative
Age (y.)		
0–29	1	0
30–39	1	7
40–49	9	4
50–59	20	50
60–69	16	51
70–	1	7
FEV (l)		
–0.6	13	18
0.7–0.9	27	49
1 0–1.2	6	49
1.3–1.5		23
Functional group		
A	6	29
B	29	84
C	13	18
Non-evaluable	0	8

mortality compared to a "normal" population showed that mortality was 6 8 times the expected in the younger group and .2 times the expected in the older group (Table III) This result, in spite of the generally better lung function in the younger men shows that when the pulmonary obstruction is present in young age the relative increase in mortality is higher than when it occurs at a higher age

Leiner et al (16) reported that age had a direct relationship to death rate A decreased maximal breathing capacity in their groups above and below 60 years increased the death rate but the relative mortality in their groups is not evaluated Simpson (24) reports only the higher risk among younger patients, but it is not possible to see whether the groups are otherwise comparable Medvei and Oswald (18) showed a relatively increased mortality among younger patients than among older in spite of a higher degree of airway obstruction compared to older patients

When ECG signs of cor pulmonale were present, the survival was considerably reduced even when higher degree of severe airway obstruction in the patients with cor pulmonale was considered The relatively small number of patients with cor pulmonale does not allow comparison within FEV groups

Table VI Patients dead after 3 years

N	Category	Dead (%)	S D	×S.D
187	All patients	29	3.3	22–36
	FEV			
31	–0 6	49	9 0	31–67
75	–0.7–0.9	35	5.5	4–46
56	–1 0–1	18	5.2	8–28
25	–1 3–1 5	8	5 4	0–19
	Functional group			
31	C	55	8 9	37–73
113	B	27	4.2	19–35
35	A	17	6.4	4–30
62	B+FEV –0 9	37	6.1	25–49
51	B+FEV 1 0–1 5	14	4.8	4– 4
63	Men >60 y	42	6	30–54
70	Men <60 y	25	5 1	15–35
48	Cor pulmonale	53	7	39–67
139	Without cor pulm.	21	3.5	14–28

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THE INFLUENCE OF PORTOSYSTEMIC SHUNT OPERATION ON IMMUNOGLOBULINS AND ESCHERICHIA COLI ANTIBODIES IN PATIENTS WITH CIRRHOSIS OF THE LIVER

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Abstract Serum γ-globulin levels have been determined by paper electrophoresis in 54 patients with cirrhosis of the liver before and after establishment of portocaval shunt. As control group 43 patients operated on for cancer of the lung were studied in the same way A significant average increase from 18.6 to 23 0 g/l in the cirrhotics was found, whereas no increase in the control group was observed. Serum immunoglobulins and antibodies to E. coli 0 group antigens were studied in 31 patients with cirrhosis and a portocaval shunt and 128 patients with cirrhosis without portocaval shunt. Significantly higher levels of IgG and E. coli antibodies were found in the patients with a portocaval shunt. It is proposed that these observation can be explained by the change in liver circulation brought about by the operation. If the cirrhotic liver has retained some of the ability of the normal liver to inhibit immunogens as previously proposed by us, the observed changes in immunoglobulins and E. coli antibodies should be expected.

Serum antibodies to common intestinal microbes exist in larger quantities in cirrhotics than in patients not suffering from liver disease (7 20) This has been explained by the failure of a diseased liver to inhibit immunogens absorbed from the gut thereby causing an increased antibody production against these immunogens. This failure could be caused by abnormalities in the liver circulation (5 6 7) or by abnormalities in the Kupffer cells of the liver sinusoids (5 7 20). The hypothesis is supported by experimental observations demonstrating an ability of the normal liver to inhibit immunogens (2 8 9 11 13) If the hypothesis is correct, it should be possible to demonstrate a rise in γ-globulins in cirrhotics in whom a portocaval shunt has been established. One study showing a rise in γ-globulins in patients following a portocaval shunt has been published (10) and we have found that antibodies to E. coli are more numerous in cirrhotics with a portocaval shunt than in cirrhotics without shunt (7) Experimental portocaval shunt in the rat is followed by an increase in serum γ-globulin (4 14 16) and also in antibodies to rat E. coli lipopolysaccharide (16)

In order to extend the observations in humans the following two studies were undertaken. 1) A retrospective study of γ-globulin levels in 54 cirrhotics before and after establishment of a portocaval shunt.) A study of immunoglobulin levels and of E. coli 0 group antibodies in 31 cirrhotics with a portocaval shunt and in 128 cirrhotics without shunt.

PATIENTS AND METHODS (STUDY 1)

During the period 1957–71 169 operations were performed at two municipal hospitals in Copenhagen, establishing portosystemic shunt in patients with cirrhosis of the liver The diagnosis was proven by means of pre- or peroperative biopsy In all cases the indication for the operation was haemorrhage from oesophageal varices.

Fifty-four patients (27 men, 27 women) with mean age of 56 years (range 34–82) ere selected from those operated on according to the following criteria. 1) Postoperative survival exceeding 1 weeks without recurrence of haemorrhage 2) No registered infection complications during the pre- (4 weeks) or postoperative (12 weeks) periods. 3) Serum γ-globulin concentration determined at least once during the pre (4 weeks) and postoperative (12 weeks) periods respectively 4) Preoperative serum γ-globulin concentration determined

Table I *Serum albumin and γ-globulin in patients with cirrhosis of the liver before and after portosystemic shunt operation and in patients before and after thoracotomy for pulmonary cancer*

	No. of pats.	Albumin (g/l)		γ-globulin (g/l)	
		Mean	S D	Mean	S D
Cirrhotics					
Preop.	54	34 4	6 0	18.6	7 0
Postop (weeks)					
1 3	46	32.3	4 9	20.2	4 9
7–9	35	32.3	5 8	23 0	8 1
Controls					
Preop	23	38.3	3 6	12.6	2.9
Postop. (weeks)					
1–3	23	34 6	4 1	13 6	2.5
7–9	23	40 1	4	13.3	2.4

prior to blood and plasma transfusions. The types of portosystemic shunt were side-to-side portocaval (33 cases) end-to-side portocaval (19 cases), and splenorenal (2 cases)

As a control group 23 patients were selected from the Department of Thoracic Surgery operated on for cancer of the lung. These controls were chosen because the operation in these cases was without direct influence on the portal circulation The controls were consecutive, but cases with postoperative infections were excluded. In all patients the amounts of blood and plasma ... during the pre- and postoperative period registered The serum albumin and serum γ-... lin concentrations were determined by paper ...lectrophoresis before and after the operation.

RESULTS (STUDY 1)

In Table I pre and postoperative serum albumin and γ-globulin concentrations are indicated for the 54 cirrhotics and the 23 controls The postoperative values are divided into two groups according to the time when the analyses were performed but all patients have been studied at least once postoperatively In the cirrhotics the decrease in albumin concentration is significant ($p<0.05$ at 1–3 weeks $p=0\ 05$ at 7–9 weeks *t*-test) The rise in γ-globulin concentration is highly significant at 7–9 weeks postoperatively ($p<0.005$) whereas the rise observed at 1–3 weeks is not significant.

When the 28 cirrhotics in whom the analyses were performed twice postoperatively are studied no significant change in albumin concentration is found and the rise in γ-globulin concentration is significant both at 1–3 weeks ($p<0\ 05$) and at 7–9 weeks ($p<0\ 0025$) In the controls a transient decrease in albumin concentration is observed at 1–3 weeks ($p<0\ 005$) whereas no significant change in γ-globulin has been found.

Transfusions given to the 54 patients in the pre per and postoperative periods were calculated as plasma 1 3 l (median) varying from 3.0 to 24.2 l The control patients received 1 1 l (median) varying from 0 to 3 0 l It is evident that the cirrhotics received much larger amounts of blood and plasma than the controls with a great variation in the quantities No significant difference between the average γ-globulin increments in cirrhotics receiving less than 7 4 l plasma (25% percentile) and those receiving 15.6 l (75% percentile) or more can be demonstrated No significant correlation *between the amount of plasma transfused and the* γ-globulin rises is found in these patients. The average rise in γ-globulin concentration in patients having end-to-side portocaval shunts is equal to that observed after side-to-side portocaval shunts

PATIENTS AND METHODS (STUDY 2)

In this study 31 patients with cirrhosis and a portocaval shunt and 128 cirrhotics without shunt were investigated. The diagnosis was established in all patients by liver biopsy Alcoholic and non-alcoholic cirrhosis were equally represented in the groups with and without shunt. Immunoglobulin levels were determined by electrophoresis in antibody-containing agarose gel (Laurell's rocket" method (15)) and E. coli 0 group antibodies by agglutination titration as described elsewhere (7). Each serum was tested with 10 common 0 group antigens. A titre of 1 40 or higher was considered positive The result of the E. coli antibody determinations is indicated as the number of positive reactions per serum Some of the results have been published in a study of E. coli antibodies in alcoholic and non-alcoholic liver diseases (18)

RESULTS (STUDY 2)

Table II shows the results of the coli antibody and immunoglobulin studies. The average IgG and IgA values are higher in the patients with a portocaval shunt than in those without. The difference is significant with regard to the IgG values ($p<0\ 0025$ *t*-test) whereas the difference between the IgA values is not significant ($0\ 10<p<0\ 15$) The average number of positive E. coli 0 group antibodies per serum is higher in the patients with a portocaval shunt than in those without. The dif

Table II. *Antibodies to E. coli 0 antigens and IgG IgA and IgM (g/l) in sera of patients with cirrhosis of the liver with and without portocaval shunt*

	No of pats	No. of pos. antibody reactions		IgG		IgA		IgM	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
With shunt	31	4.6	3.0	24.1	6.8	5.[illegible]	2.5	1.41	0.60
Without shunt	125	3.3	2.9	19.0	8.4	4.6	3.3	1.45	1.48

ference is statistically significant ($p<0.025$ Wilcoxon rank sum test). The intensity of the antibody reactions is the same in the two groups of patients. After exclusion of all prednisone-treated patients (35 patients in the group of cirrhotics without portocaval shunt) the difference with regard to IgG levels and coli antibodies remain significant. There is no difference in the Ig values or number of positive coli reactions per serum between alcoholic and non-alcoholic patients with a portocaval shunt.

DISCUSSION

In the first study the figures shown in Table I demonstrate that a small decrease in albumin concentration and a rise in γ-globulin concentration follow the establishment of a portocaval shunt. A transient decrease in albumin concentration occurred in the control patients operated on for cancer of the lung. The decrease in albumin concentration is similar to alterations found in surgical trauma (17, 21, 22) and is caused by a temporary internal loss of albumin into the wound oedema and by a temporarily decreased rate of synthesis (17). We believe that the rise in γ-globulin is due to the alteration in the liver circulation brought about by the portocaval shunt. However several important sources of error have to be considered before this result can be accepted. It is not likely that the rise in γ-globulin concentration is an unspecific response to the operation trauma, since there is no rise in the control group and reports in the literature state that there is no increase in γ-globulin in relation to cholecystectomy (22) and gastrectomy (21). Most important as a source of error are the large amounts of blood and plasma given, larger than those given to the controls. Theoretically the plasma infused could cause an increase in γ-globulin for two reasons: retention of γ-globulin in the vascular space because of its large molecule and retention because of its relatively slow catabolic rate. However the increase in γ-globulin is not related to the amount of plasma given. This would be expected if retention of transfused γ-globulin was the cause of the increase observed.

Furthermore it has been demonstrated in patients with anaemia that transfusions in amounts corresponding to 35% of the blood volume do not change serum γ-globulin concentrations (1). These observations together with our own observations in the controls, also speak against the possibility that antigens in the blood transfusions are the cause of the γ-globulin increase observed. Finally changes in blood volume as possible explanation are not likely as the albumin in our patients is either unchanged or decreasing transiently.

We therefore conclude from this part of our study that the γ-globulin increase observed is related to the change in the liver circulation established by the portocaval shunt. The γ-globulin level was already high before the operation, but the portocaval shunt itself brought about a further increase of the level.

The second part of our study demonstrates that patients with cirrhosis and a portocaval shunt have higher IgG levels and more E. coli 0 group antibodies than cirrhotics without shunt and thus supports the first part of the study.

As far as we know there is only one publication in the literature concerning this problem. Esser (10) has demonstrated an average increase in serum γ-globulin in 107 patients from 1.83 g/100 ml before operation to 2.24 g/100 ml 4 weeks after operation, an increase of the same order as in our material.

The effect of the portocaval shunt on the level of coli antibodies and immunoglobulins can prob-

ably be explained in two different ways. With regard to antigens absorbed directly into the portal blood the fact that the portal blood bypasses the liver in the first circulation may well be of importance for the antibody production These antigens reach antibody-producing tissues before they reach the greatest part of the RES of the body the Kupffer cells in the liver which probably act as inhibitors of the immunogenicity of the antigens (11 13) With regard to other antigens absorbed into the intestinal lymphatics and the thoracic duct and therefore reaching the liver via the general circulation the explanation cannot be so simple (6) The intestinal microbes absorbed probably belong to this category (1) Here we must presume that the low liver blood flow in patients with a portocaval shunt is the important factor (3 19). The function of the RES of the liver must be much depressed because of the low blood flow and this is probably the explanation of the high level of the coli antibodies

The high immunoglobulin values may be explained as the sum of these two effects the bypassing of the liver and the low liver blood flow The high level of immunoglobulins before the operation is probably due to the fact that these patients already have a pronounced collateral circulation. The increase in the level of the immunoglobulins may reflect the fact that the shunt be s now even greater and the liver blood flow aller

The fact that alcoholism does not influence the amount of antibodies may be due to the overwhelming influence of the change in circulation so that the smaller effect of alcohol cannot be observed under these circumstances

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EFFECT OF ORPHENADRINE ON TOTAL THYROXINE

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Abstract Three clinically euthyroid patients with parkinsonism were found to have raised levels of protein-bound thyroxine (T_4(D)). It was demonstrated that treatment with orphenadrine (Disipal®) caused an elevation of T_4(D) up to more than 100%. The increase was dose-dependent and the underlying mechanism remained unknown.

The assay of total thyroxine (T_4(D)) is based on the specific binding properties of thyroxine-binding globulin (TBG) (5). In contrast to protein-bound iodine (PBI) the determination of T_4(D) is not influenced by the administration of iodine or mercury However it has been shown that some drugs can induce pathological levels of T_4(D) in euthyroid patients. Clofibrate and estrogenous compounds increase the binding capacity of TBG which results in elevation of T_4(D) (1 5) Diphenylhydantoin decreases T_4(D) by competing for the binding sites of TBG (4).

In three patients with parkinsonism we have observed that treatment with orphenadrine hydrochloride (Disipal®) can increase the serum levels of T_4(D).

The patients were clinically euthyroid and had normal values of T_4(D), determined according to Murphy and Jachan (8), and of RT_3U (uptake of ^{125}I-T on protein-coated charcoal) measured as described by Herbert et al. (3). During treatment the T_4(D) level increased in all three patients and this increase seemed to be dose related (Fig. 1). The RT_3U values remained unchanged. After withdrawal of Disipal the T_4(D) values returned to normal. TSH was normal when measured with and without medication in two of the patients. These two patients (nos. 1 and 3) had also normal urinary excretion of estrone and estradiol during treatment. Elevated TBG values, measured according to Nielsen et al. (7), were found in patient 1 during treatment (222 and 186% normal level 100%) and a slight increase of TBG (117–131%) was noticed in patient 3 during treatment.

The addition of orphenadrine citrate to plasma from a healthy volunteer in concentrations of 1 5 10 50 and 100 µg/ml plasma did not increase the T_4(D) level.

DISCUSSION

It appears likely that the elevation of T_4(D) in these three patients was caused by the treatment with Disipal® They had normal T_4(D) levels initially but raised levels during treatment with Disipal. Furthermore, normalization of T_4(D) was seen after withdrawal of the medication The T_4(D) elevation was dose-related in all patients (Fig. 1)

It appears that Disipal does not influence the T_4(D) assay since the T_4(D) was unchanged in all samples after addition of 1–100 µg orphenadrine. The plasma levels of orphenadrine in pa

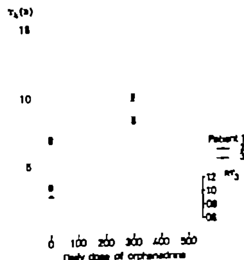


Fig. 1 T_4(D) and RT_3U in three patients treated with orphenadrine.

tients treated with this compound are not known but it is unlikely that they exceed 100 μg or phenadrine/ml plasma at the dosages used

The increased TBG levels in patient 1 might explain part of the T_4(D) elevation but this is not in agreement with the minor increase of TBG in patient 3 Another possibility of the effect of orphenadrine on T_4(D) would be that the compound increases the binding capacity of TBG However the RT_3U levels did not decrease Thus the mechanism remains obscure

Hansen and Siersbæk Nielsen (2) studied the PBI levels in 50 patients treated with perphenazine Nine of their patients had PBI levels higher than 8 μg/100 ml It is remarkable that five of these nine patients were also on orphenadrine treatment It is not unlikely that the PBI elevation in these patients was caused by orphenadrine

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PRIMARY AMYLOIDOSIS WITH POLYNEUROPATHY

Some Aspects on the Histopathological Diagnosis Ante Mortem based on Studies of Biopsy Specimens from 30 Familial and Non-familial Cases

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Abstract. Biopsy specimens from 30 patient with primary amyloidosis and polyneuropathy have been reviewed with regard to the histopathological methods used and to the kind of tissues examined The importance of using modern histopathological methods to establish the amyloid diagnosis was demonstrated. Bi opsy of the rectal mucosa of 18 patients was positive for amyloid in all. Skin biopsy was positive in 22 of 25 patients. I one patient the first kin and rectal biopsies were negative: new specimen after one year and three years, respectively were positive for amyloid. In all 30 patients amyloid deposits were found either in the rectal mucosa or in the skin Rectal and skin biopsies are recommended as valuable procedures to confirm the clinical diagnosis of primary amyloidosis with polyneuropathy

During the last few years about 50 cases of primary amyloidosis with polyneuropathy most of them familial have been diagnosed in the north of Sweden. In the first cases some difficulties were encountered in proving histologically the occur rence of amyloid deposits (7). As a conclusive diagnosis of amyloidosis depends on histopathological verification, we have found it valuable to review the results of our examinations with regard to the kind of tissues investigated and to the methods used The main purposes of this study were to see whether skin biopsy was as valuable among our patients as it is reported to be in Portugal (14), to review our results of rectal biopsy and to compare the results of the skin and rectal biopsy procedures. The present report will also serve to illustrate the importance of using modern sensitive histopathological methods (11 13) to demonstrate the amyloid deposits also in this type of amyloidosis

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MATERIAL

The material consisted of 30 patients 1 men and 9 women. Three patient were found during a genealogical investigation of the disease The others were dmitted to hospital because of more or les distinct ymptoms and signs of polyneuropathy Other clinical manifestations were gastrointestinal and rinary bladder disturbances, heart affection and isual disturbances because of itreous opacities In 21 patients, 13 men and 8 women, the disease was found t be familial The families are indicated by capital letters, from A to G. To each familial case is added number Nine patients considered sporadic so far are indicated by capital letters only from H to P Reports concerning some of these cases have appeared previously (2, 3 4 9) The histological confirmation of amyloid deposits was made ante mortem in 26 cases. In three cases (A.5 A.7 B.2) it was not made until after the patient death at reexamination of biopsy specimen taken before death. I one case (H) the diagnosis was proved first t autopsy In no case was chronic inflammatory disease myelomatosis or tumour detected.

Data concerning sex age at biopsy and duration from onset of clinical symptoms are given in Table I The clinical evaluation of the neuropathy which appeared first and most markedly in the legs also shown in Table I It is graded semiquantitatively as in preceding report (3) In 15 patients the neuropathy in the legs was marked (+++) moderate (++) in 11 and slight (+) i 4 Clinically no significant differences were found when repeated xaminations were per formed.

METHODS

In all patients biopsy was performed ante mortem for histological examination. Skin specimens were taken as

Table I *Examination of amyloid deposits in biopsy specimens from 30 cases of primary amyloidosis with polyneuropathy*

Case	Sex	Age at biopsy (y)	Duration of symptoms (y)	Clinical grading of neuropathy in legs[a]	Tissue specimen	Examination of amyloid deposits: Methyl violet and van Gieson's stain (Positive)	(Negative)
A.1	♂	66	16	+++	Nerve skin rectum, muscle liver kidney tongue gingiva	Nerve	Skin, rectum, muscle liver kidney tongue, gingiva
A.2	♀	63	10	+++	Nerve skin, muscle	Nerve	Skin muscle
A.5	♂	70	24	++	Muscle urinary bladder		Muscle urinary bladder[b]
A.6	♂	61–64	3–6	+++	Rectum muscle (61) skin (64)		Rectum muscle[b] (61)
A.7	♂	68	18	+++	Skin rectum muscle liver gingiva		Skin muscle, rectum, liver gingiva
B.1	♀	61–61	4–5	+++	Nerve rectum (60), skin rectum (61)	Nerve	Rectum (60)
B.2	♀	68–70	5–7	+++	Nerve skin, rectum muscle (68), salivary gland (70)[b]	Salivary gland (70)[b]	Nerve, skin, rectum, muscle (68)
B.3	♂	43	3	++	Skin, rectum		
B.6	♂	57	6	++	Nerve skin jejunum		
B.7	♀	35	2	+	Skin		
B.8	♀	40–43	1 3	+	Rectum (40), skin (42), skin rectum (43)		
B.9	♂	65	5	++	Skin		
B.10	♀	35	2	+	Skin		
C.2	♂	32 34	3–5	+++	Skin muscle jejunum (32) skin (34), nerve skin rectum (34)		Skin,[b] muscle jejunum[b] (32)
D.1	♂	66	3	+++	Rectum		
D.2	♂	68	5	+++	Rectum		
D.3	♀	60	13	+++	Rectum		
E.1	♀	63	9	++	Skin rectum muscle		
E.2	♂	70	14	+++	Nerve skin		
F.1	♂	62	6	++	Skin rectum		
G.1	♂	57 59	1 3	++	Skin (57) nerve rectum (58) skin (59)		Skin[b] (57)
	♂	51	2	+++	Liver jejunum		Liver jejunum[b]
	♀	64–66	7–9	++	Skin rectum (64) rectum (65) rectum (66) skin rectum (66)		Rectum (64)
	♂	38	2	++	Skin, rectum		
K	♂	54	4	++	Skin		
L	♂	57	7	+++	Skin		
M	♂	69	4	+++	Nerve skin skin		
N	♂	61	15	++	Skin		
O	♂	73–75	3–5	+	Rectum (73) skin (75)		
P	♂	78	10	+++	Skin rectum		

[a] + = slight, ++ = moderate, +++ = marked [b] Amyloidosis clinically not suspected at the time of examination.
Figures within parentheses = patient's age at the time of repeated biopsy

small punch biopsies. The inner diameter of the rotating cylinder knife used was 2 mm. In general we have performed the skin biopsies in the distal part of the lower leg. We have tried to perform the biopsy adjacent to hair follicles in order to get arrector pili muscles in the specimens. Biopsy of the rectal mucosal membrane was made with a pair of conventional biopsy forceps. Surgical biopsies were made of the sural nerve and of striated muscles of the lower leg. Tissue specimens were fixed in 10% formalin and routinely embedded in paraffin. Two examination procedures were used:

(I) Before the alkaline Congo red method had been introduced in our laboratory sections routinely stained with van Gieson's stain or with hematoxylin and eosin were examined for the presence of deposits that could represent amyloid. If such deposits were present staining with methyl violet was also performed. All specimens were later reexamined according to procedure II.

(II) After the alkaline Congo red method (13) had been introduced as a routine method in our laboratory all biopsy specimens from cases supposed to suffer from amyloidosis were investigated according to that method. Sections stained by the alkaline Congo red method were examined light-microscopically both in ordinary and in polarized light (11).

Alkaline Congo red and polarized light (Positive)	(Negative)
Nerve, skin rectum, muscle, tongue gingiva	Liver kidney
Nerve, skin, muscle	
Urinary bladder	Muscle
Rectum, muscle (61), skin (64)	
Skin, rectum, muscle	Liver
Nerve rectum (60) skin, rectum (61)	
Nerve skin rectum, muscle (68), salivary gland (70)	
Skin, rectum	
Nerve, skin	Jejunum
Skin	
Skin, rectum (43)	Rectum (40) skin (42)
Skin	
Skin	
Skin, muscle Jejunum (32) skin (34) nerve, skin, rectum (34)	
Rectum	
Rectum	
Rectum	
Rectum	Skin, muscle
Nerve, skin	
Rectum	Skin
Skin (57), nerv rectum (58) skin (59)	
	Liver jejunum
Skin, rectum (64), rectum (65), rectum (66), skin, rectum (66)	
Skin, rectum	
Skin	
Skin	
Nerve, skin, skin	
Skin	
Rectum (73)	Skin (75)
Skin, rectum	

RESULTS

The various *tissue* specimens taken for microscopic examinations and the results are shown in Table I. Especially in some of our first patients (A.1 A.7 B.2, C.2) several biopsies were performed.

(I) By method I deposits of an amorphous picrinophil substance were found in the sural nerve of cases A.1 A.., and B 1 and in sections from a submandibular salivary gland of case B.2. The substance showed a metachromatic reaction with methyl violet. Otherwise examinations with these staining methods were negative for amyloid in many specimens (Table I)

(II) Light-microscopical examination, both in ordinary and polarized light after staining with alkaline Congo red revealed amyloid deposits in *most of the specimens (Table I)* Of 82 specimens examined in that way amyloid was detected in 69 No amyloid was found in 4 biopsies of the skin (E.1 F 1 0 and the first skin biopsy of B.8) No amyloid was found in 3 biopsies of the liver (A.1 A 7 H) 2 of striated muscles (A.5 E.1) of the jejunum (B:6 H) 1 of the kidney (A.1) or in the first rectal biopsy of patient B:8

The clinician assumed a diagnosis of amyloidosis in 8 patients from whom 23 biopsy specimens were examined by both methods With methyl violet and van Gieson s stain amyloid deposits were detected in 3 specimens from peripheral nerves *only (vide supra) On examination* with polarized light after staining with alkaline Congo red however amyloid was found to be present in 19 of the 23 specimens (Table I) No amyloid was detected in biopsy specimens from the liver (A.1 A.7 H) or from the kidney (A.1).

Amyloidosis was clinically not assumed at the time when the examination of 10 biopsy specimens from 7 patients was performed (Table I) By method I amyloid was found in the salivary gland of case B.2 only (This biopsy was performed because of a tumourlike enlargement of a submandibular salivary gland.) Subsequent reexamination by method II revealed amyloid deposits in 7 additional specimens of 5 patients (Table I).

Biopsy was performed of the *rectal mucosal membrane* of 18 patients Amyloid was found in all of them. Amyloid was detected in altogether 22 of 23 rectal biopsies The deposit of amyloid was found to be located particularly in the muscular layer of the mucosa (Fig. 1). In specimens containing parts of the submucosa amyloid appeared *in vessel walls and in the perivascular* connective tissue

Thirty-one specimens of *skin* biopsy from 25 patients were examined by polarization microscopy after staining with alkaline Congo red Amyloid deposits were detected in 27 specimens from 22 cases It was found in 3 of 4 patients with slight



Fig 1 Section from the rectal mucosa of case B·1 In partly polarized light each area containing amyloid appears as bright green (white in the photomicrograph) and red (black) patches. Most of the amyloid i the muscular coat of the mucosa (*m.m*) Is found in its deeper parts Two arrow heads indicate the largest deposit located there. Small deposits (arrows) can also be seen in the upper parts, close to the crypts. Alkaline Congo red stain and partly polarized light. ×135

and in 8 of 10 with moderate manifestations of neuropathy in the legs at the time of examination. In 11 patients with marked signs of peripheral neuropathy amyloid was found in the skin of all The amyloid deposit was often found to be adjacent to the arrector pili muscles (Fig.)in relation to the sweat glands and in vessel alls

Both skin and rectal biopsies were performed on 20 occasions in 15 patients Amyloid was found in 19 of the 20 rectal and in 16 of the 20 skin biopsy specimens The patient with both skin and rectal biopsies negative for amyloid at the first examinations (B.8) had only a slight degree of peripheral neuropathy

We performed biopsy of the *sural nerve* in 9 patients Amyloid was detected in all specimens (Table I) Seven of these patients had marked and two moderate peripheral neuropathy In the nerves the amyloid deposits were located especially in the *endo-* and *perineurium* often appearing as rounded masses (Fig. 3).

Amyloid was found in biopsy of the *jejunum* of 1 case whereas no amyloid was detected in such specimens of . other cases (Table I) In some other patients e g. A.7 B 7 and J repeated trials were made to get the biopsy instrument through the pyloric region but all were unsuccessful.

In 8 patients biopsy was performed of *striated muscles* (Table I) Varying degrees of neurogenic atrophy of the musculature were observed. Deposits of amyloid were found in 6 patients who all had marked clinical signs of peripheral neuropathy The deposits, however were scanty appearing chiefly in the vessel walls and in the nerves The two patients without any amyloid in the striated muscle specimens had more moderate manifestations of peripheral neuropathy

Liver biopsy was examined in 3 cases only In none of them could any amyloid be detected.

COMMENTS

The preferential reaction of amyloid to crystal violet and methyl violet, termed metachromasia, was found to be a useful staining method for tissue sections (7) So was staining with Congo red (6) which gives amyloid a pink colour Sometimes however it may be difficult to demonstrate amyloid with these staining methods Therefore it was a great step forward when it was observed that amyloid gave a green birefringence after *staining with Congo red when viewed in the* polarization microscope (11) By staining with alkaline solution of Congo red (13) the examination achieved some advantages It seems to be a really



Fig 2 Part of biopsy specimen from the skin of case A.2. Amyloid (black) forms the border of arrector pili muscles (*p*) Alkaline Congo red stain. ×129

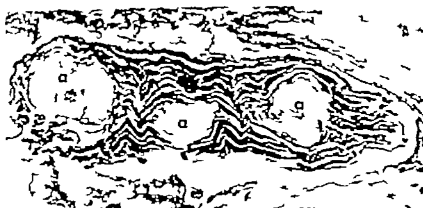


Fig. 1. Section from the sural nerve of case A... Three rounded patches of amyloid ( ) are show... Van Gieson stain 165

sensitive method for demonstrating even small amounts of amyloid. Our study shows that it is also of importance for the examination of biopsy specimens from patients suffering from amyloidosis with polyneuropathy.

Examination of biopsy specimens from the rectal mucosa often reveals amyloid accumulation in systemic amyloidosis (8). In preceding reports on familial amyloidosis with polyneuropathy, however, the results of rectal biopsies have been described only infrequently (14). Our observations show that biopsy of the rectal mucosa is a valuable procedure to demonstrate amyloid deposit in this type of amyloidosis. In agreement with reports on the distribution of amyloid deposits in the stomach and small intestine (12, 15) we found that the amyloid substance in the rectal mucosa was located especially in relation to the muscular layer. This part of the mucosa thus has to be included in the biopsy specimen in order to get relevant material.

Skin biopsy has been reported to be a useful diagnostic procedure in familial amyloidosis with polyneuropathy. In 24 biopsy specimens of the skin from 23 patients in Portugal amyloid was detected in 23 (14). In our cases, too, examination of skin biopsy specimens was found often to reveal amyloid deposits. In agreement with reports from Portugal (14) we often found amyloid deposits located adjacent to the arrector pili muscles, in relation to sweat glands and in vessel walls.

When comparing the results of examinations of rectal and skin biopsies in our material, amyloid was found almost as frequently in the skin as in the rectal specimens. As the symptoms and signs of peripheral neuropathy in our patients appeared first and most markedly in the lower limbs, we think that skin biopsy of the l wer part f the leg may be an appropriate procedure. It ...asy to perform and if it is negative in cases ... ally supposed to have amyloid deposits such ... psy can easily be repeated. Biopsy of the rec... m... cosa is a useful alternati e or supplemen... procedure.

To judge from our observations it is obvious that biopsy of a peripheral nerve also may be useful alternative. In cases with marked neur... pathy and with disturbances of the sensibility this procedure is harmless. In such cases, however, biopsy of the skin and rectum may be expected to reveal amyloid as well. In patients with only discrete symptoms of neuropathy, nerve biopsy may be more conclusi e than biopsy of the skin or rectum. As far as we know, however, this question has not been studied and has to be left open.

In Portugal, where gastro ntestinal disturbances also occur frequently among patients w th this disease (5), biopsy of the stomach (17) and of the small intestine (15) has been reported to re eal amyloid deposits. Because of the gastric neuropathy with disturbance of motility and dealy in emptying of the stomach we found it difficult to pass a biopsy capsule beyond the pyloric region. Therefore, in this study we were able to get biopsy specimens of the jejunum in only a few cases. Biopsy of the gastric mucosa was not performed.

A progressive atrophy of striated muscles, especially in the distal parts of the limbs, is typical of this disease. Amyloid may appear in the walls of

larger blood vessels located interstitially in the musculature It is apparent from this study however and from our examination of autopsy cases (10) that, even in the presence of advanced muscular atrophy no or only minor amounts of amyloid are to be found in the rather small specimens usually taken at skeletal muscle biopsies

As we have found at autopsy examinations (10) and as also reported from Portugal (14) amyloid accumulation in the liver is usually scanty in this form of amyloidosis It appears in the vessel walls of the portal triad only In none of our 3 liver biopsies could amyloid be detected The amyloid involvement of solid viscera including the liver however may be variable and more extensive in other families Thus in a report from Iowa, USA (1) concerning familial cases of amyloidosis with polyneuropathy nephropathy and peptic ulcer the examination of biopsy specimens of internal parenchymatous organs was said to be an appropriate procedure

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TREATMENT OF ACUTE LEUKEMIA IN ADULTS

Results with a Combin tion of Potent Cytotoxic Drugs

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Abstract A total of 66 adult patients with acute leukemia were seen in the 2-year period May 1971 May 1973 I 4 patients the rate of progression was judged to be slow and they were therefore left untreated. Two patients with acute myeloid leukemia (AML) died 12 hours and 7 days, respectively after start of treatment and are excluded s non-evaluable. The remaining 60 treated patients include 8 acute lymphatic leukemias with 7 remissions, 6 stem cell leukemias with 5 remissions, and 46 AMLs with 25 remissions. Treatment of 26 patients with AML started more than 12 months ago 16 of them went into remission and have a median survival of 14 months, compared to 4 months in the 10 not obtaining remission. Five AML patients are in complete remission, 28, 27 26 23 and 22 months, respectively after start of therapy

Until about 5 years ago the results of treatment of patients with acute myeloid leukemia (AML) were very poor and many hematologists therefore only gave supporting treatment to AML patients. During the last 5 years treatment with combinations of daunorubicin, cytosine arabinoside (and sometimes other drugs in addition) have in some centres resulted in remission rates of more than 50% (1 2, 5)

In May 1971 we started treating adults with acute leukemia with a combination of potent cytotoxic drugs Our combinations of drugs were only a sl ght modification of those of Spiers (6)

MATERIAL

Our Section of Haematology receives patients referred from hospitals in all parts of Norway W have accepted for admission any patient with newly discovered acute leukemia between 15 and 60 years of age except for 3 patients who had previous severe coronary artery disease (These 3 patients were not admitted and were not given the opportunity of treatment with our combination of potent cytotoxic drugs. However they were not more critically ill than many of those admitted for treatment and might have obtained remission if they had been given the opportunity of the potent treatment.) Three patients above 60 years of age (64 64 62 years) have been treated

All patients have been admitted t our section within 2 days of referral. We have started chemotherapeutic treatment as soon as the patient has been admitted, regardless of fever etc.

Classificatio of acute leukemia

Our differentiation between acute myeloid (AML) and acute lymphoblastic leukemia (ALL) was mainly based on the appearance of the primitive cells May) Grünwald-Giemsa-stained blood and bone marrow films ( ) More than 5% promyelocytes indicated acute myeloid. (*b*) Auer rods were diagnostic of acute myeloid. (*c*) If the blasts had a free brim of cytoplasm larger than 1/3 of the area of the nucleus this suggested acute myeloid. If the free brim was les than 1/4 of the area of the nucleus it suggested ALL. (Unfortunately the free brim was often between 1/4 and 1/3 of the area of the nucleus, leaving one undecided!)

TREATMENT

AML (all variants). We generally treated for 5 consecutive days of each 2 week period during the first year and for 5 consecutive days of each 3-week period during the second year Our combinations of four drugs and our method of treatment was only slight modification of that of Spiers (6) The first six (induction) courses of treatment consisted of daunorubicin 60 mg i. on day 1 only cytosine arabinoside 150 mg i. daily for 5 days, prednisone 40 mg by mouth daily for 5 days, and either 150 mg 6-mercaptopurine or 120 mg thioguanine by mouth daily for 5 days.

The next ten (maintenance) courses consisted of six which were exactly like the induction courses, and two courses in which 60 mg daunorubicin on day 1 was replaced by 2 mg vincristine i. and 150 mg 6-mer

A

captopurine was replaced by 150 mg cyclophosphamide i v daily for 5 days The last two maintenance courses consisted of vincristine 2 mg i.v on day 1 methotrexate 7 / mg by mouth daily for 5 days, prednisone 40 mg daily for 5 days, and 6-mercaptopurine 500 mg by mouth daily for 5 days.

These ten maintenance courses were then repeated over and over again two courses containing vincristine always following after three courses containing daunorubicin

If the bone marrow became blast-free but hypoplastic the free interval between two treatment courses was sometimes increased from 9 to 16 days even during the first year of treatment.

ALL, age 15–20 years We gave vincristine 2 mg/m² i v once a week for 3 or 4 weeks prednisone by mouth 40 mg/m²/day for 4 weeks and 6-mercaptopurine 100–150 mg daily This usually resulted in remission within 3–4 weeks. The remission was then maintained with 6-mercaptopurine 100–150 mg daily Reinforcement of the remission with vincristine I was given every 3 months. When the patient obtained hematological remission CNS prophylaxis was given with 5 intraspinal injections each of 20 mg methotrexate Subsequently 20 mg methotrexate was given intraspinally twice every 3 months.

ALL, age above 20 years We used a treatment which was only slightly modified from that of Spiers (6) This regimen resembles that used for AML but the combinations of four drugs is slightly different. The first six (induction) courses of treatment consisted of vincristine 2 mg i.v on day 1 only cytosine arabinoside 150 mg i daily for 5 days cyclophosphamide 150 mg i daily for 5 days and prednisone 200 mg daily for 5 days.

The next eight (maintenance) courses consisted of which were exactly like the induction courses and courses consisting of daunorubicin 60 mg on day 1 *ytosine arabinoside 150 mg i daily for 5 days and 6-*mercaptopurine 150 mg by mouth daily for 5 days. The two remaining maintenance courses consisted of vincristine 2 mg on day 1 methotrexate 7 /₂ mg by mouth daily for 5 days, prednisone 200 mg daily for 5 days and 6-mercaptopurine 500 mg by mouth daily for 5 days.

If the bone marrow became blast-free but hypoplastic the free interval between two treatment courses was sometimes increased from 9 to 16 days, even during the first year of treatment. If the patient developed signs of paresis of the fibular muscles (unable to walk on his heels) vincristine was omitted from one course of treatment

Unclassified stem cell leukemia These patients were treated like ALL patients above 20 years of age.

Supporting care

In the initial phase we gave liberal amounts of fresh blood transfusions. Sometimes platelet-rich plasma was given for thrombocytopenia with bleeding, but we did not have facilities for granulocyte transfusions.

Severe granulocytopenic patients were kept in single rooms and visitors kept to a minimum but life island or laminar air flow technique was not available.

We did *not* use prophylactic antibiotics or bowel sterilization but we started immediately with large doses of antibiotics in febrile patients.

Criteria of remission

(*a*) Less than 5% blasts in the bone marrow and less than 10% promyelocytes. (*b*) Platelets within the normal range at least in some part of the treatment cycle. (A somewhat reduced number of granulocytes and some degree of anemia *may be caused* by toxic action on bone marrow or hemolysis, due to the cytotoxic drugs.)

RESULTS

Table I gives a survey of the 66 new cases of acute leukemia admitted during the 2 year period May 1971 – May 1973 Four of the 66 patients were *not* started on the combination of potent cytotoxic drugs because they were considered to be of the slowly progressing type not likely to benefit from it Table II groups the treated patients according to whether they obtained remission or not. Two cases have been excluded as not evaluable one died 12 hours, the other 7 days after admission Table III lists the median survival for the patients obtaining and for those not ob-

Table I. *New cases of acute leukemia in May 1971 – May 1973*

Type	No of pats	Age (y) Range	Median	Treated	Not treated
ALL	8	13–33	21	8	
Stem cell	6	26–59	43	6	
AML					
"Classical"	27	15–64	46	26	1
Myelomonocytic	18	17–69	41	18	
Erythrocytic	6	43–77	61	3	3
Hypergranular promyelocytic	1	38		1	
Total	66			62	4

Table II *Treated patients [illegible]ith different variants of AML grouped according to [illegible]emission or no remission*

	No. of pats.	Remis-sion	No re mission	Age (y) Range	Median
Treated					
ALL	8	7		13–30	18
			1	33	
Stem cell	6	5		26–59	39
			1	43	
AML (all variants)*	46	25		16–6[illegible]	40
			21	15–69	46
Total	60	37		13–62	35
			23	15–69	43
AML					
"Classical"	26	13		16–6[illegible]	38
			13	15–64	49
Myelomonocytic	17	10		18–58	41
			7	17–69	41
Erythrocytic		1		61	
			1	54	
Hypergranular promyelocytic[a]	1	1		38	
Total	46	25		16–6[illegible]	40
			21	15–69	46

Two patients who died are excluded.
Stavem (7).

taining remission Table IV shows median survival for all the treated patients regardless of whether they obtained remission or not.

DISCUSSION

It has been stated (3) that chemotherapy in AML is least likely to benefit patients whose disease processes are progressing at either a [illegible]ery rapid or very slow rate

Patients acutely and desperately ill when first diagnosed may die before they reach the leukemia centre from the local hospital. Also some centres will initially only give antibiotics and supporting treatment to desperately ill patients and only those who get over their infection etc. will be put on a chemotherapeutic regimen (3). A variable portion of the poorest risk AML patients may in these ways be omitted from the trials in larger centres like our section. This has to be kept in mind when comparing results from different hospitals. The remission rate among all patients diagnosed as AML in a general city hospital will for these reasons tend to be lower than in larger leukemia centres like ours to which patients are referred from local hospitals

The slowly progressing patients probably re present a relatively small fraction of the patients

Table III *Median [illegible]urvival for pati[illegible]nts started on treatment more than 12 months ago grouped according to remission or no remission*

	N of pats	Remis-sion	No re-mission	Median survival (mo)	Age (y) Range	Median
ALL	7	6		14	13–30	21
			1	1	33	
Stem cell	5	4		17	26–59	41
			1	2	43	
AML (all variants)	26	16		14	17–61	38
			10	4	31–64	46

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BOOK REVIEW

Environmental Health Aspects of Lead International symposium Amsterdam October 2-6 1972 1168 pp. Price c. 265 Sw cr CID EG May 1973

In Oct. 1972 a common symposium on plumbism was organized in Amsterdam by the Commission of the European Communities (CEC) and the United States Environmental Protection Agency (EPA)

Among the 300 specialists from all over the world there were physicians, biochemists, toxicologists economists and many other professions. The problems therefore could be treated from many points of view. In the seven sections there were more than 90 papers. Among the subjects treated were the presence of lead in the external milieu the question how the metal reaches the human being, the incorporation of lead in the human organism the toxicology and metabolism of lead in different animal species, cytotoxic and biochemical effects of lead in man, subclinical effects of lead and their usefulness for the diagnosis of early lead poisoning, the epidemiology of plumbism and, finally methods for the analysis of lead. There was also a round table discussion with a panel judging available data regarding the detrimental influence on the human organism.

The volume is very well edited and should become a standard work for everybody who is interested in lead problems. Most of the acute problems in the discussion about plumbism are presented in an understandable way. The language is almost entirely English. German and French papers have an English summary

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MULTIPLE JEJUNAL STRICTURES ASSOCIATED WITH MEGALOBLASTIC ANAEMIA AND MYELOPATHY

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Abstract In patient with multiple strictures chronic stasis and dilatation of the jejunum eurological defects suggestive of subacute combined degeneration of the spinal cord developed over 10-year period. I spite of optimal substitution therapy with vitamin B_{12} the bone marrow was megaloblastic, which was ascribed to f late deficiency resulting from malabsorption of folic acid. A possible association between the neurological lesions and the folat deficiency was not established, as there may also have been deficiency of other nutritional factors of significance for nervous tissue. Malabsorption carries gra prognosis in this condition, but in the case presented all symptoms of malabsorption were relieved by surgical removal of the jejunal strictures.

The association between jejunal strictures and "pernicious anaemia" was first described by Faber (10) in 1897 So far relatively few observations of this stricture anaemia" have been reported In 1969 Chanarin (6) traced 51 cases in the literature and an additional case was published in 1971 (2).

The diagnosis is difficult to establish In many cases the patient is young and has suffered from dyspepsia for several years Nausea colicky pain in the epigastrium abdominal rumbling and projec tile vomiting are conspicuous symptoms Steatorrhoea may be a pronounced feature. Weight loss usually occurs, and in 25% of the patients neurological defects develop (5)

Meulengracht (14) observed that liver therapy could bring about remission of the anaemia, and most of the patients in his study seemed to suffer from vitamin B_{12} deficiency Folate deficiency may also be present (2). Surgical correction of the anatomical abnormality leads to remission of the megaloblastic anaemia, but owing to the poor general condition of the patients the operative mortality is high (5)

The pathogenesis of this stricture anaemia is unknown Faber (10) assumed that toxin produced by the stagnant intestinal contents was the cause of the anaemia—a view which was subsequently supported by Thompson and L gley (17) who suggested that a toxin produce by intestinal bacteria led to haemolysis which n turn resulted in itamin B_{12} and folic acid defi ciency Cameron et al (5) presumed that the testinal stagnation inhibited the absorption of n or more haemopoietic factors and Halsted et al (12) explained the megaloblastic anaemia by vitamin B_{12}-consuming bacterial flora in the stag nant intestinal contents.

In the case described here malabsorption of folic acid and vitamin B_{12} were outstanding features which however could be relieved by surgical correction of the anatomical defect

CASE REPORT

The patient, male, then aged 16 years, was admitted to hospital for the first time in 1960 with complai t of persistent diarrhoea. I childhood he had been in good health. The dyspeptic symptoms had begun about 1 months previously with colicky epigastric pain especially after meals. Occasionally projectile omiting—without preceding nausea—occurred, resulting in relief of the pain. The attacks were episodic with free periods of 1–2 months. No weight loss had occurred. During the hospital stay the stools were voluminous and loose but did not ppear to be fatty Stool examinations for blood were negative, and barium meal studies did not reveal any abnormalities

In view of the indefinite findings no treatment was given and the condition remained largely unchanged until 1966 when the patient was readmitted with diarrhoea and borborygmi.

The stools were now suggestive of steatorrhoea, and an examination showed excretion of large amounts of

Fig. 1. Survey of data and therapeutic measures.



Fig 2. Barium meal study (1970) showing multiple strictures and dilatation of the jejunum.

fat in the faeces (>30 g/24 h). A before the patient complained of colicky pain which wa relieved by vomiting His nutritional condition was poor height 175 cm, body weight 48 kg. Decreased absorption of lactose glucose and D-xylose and slight anaemia were revealed, but the bone marrow was normal, and so was the vitamin B concentration. The FIGLU test (formiminoglutamic acid i 5-hou urine specimen after histidine load) showed increased values, indicating folate deficiency (Fig. 1). The Schilling test with and ithout intrinsic factor yielded pathologically low values. Serum calcium and serum protein concentrations were depressed. N episodes of tetania were observed. Barium meal studies revealed retention after 12 hours, but no gastric ulcers. The intestinal motility was depressed and the duodenum was found to be dilated.

Malabsorption was thus present, and even though the clinical picture was not quite characteristic gluten-free diet as instituted on the suspicion of coeliac disease (Fig. 1) together with substitution therapy with injections of itamins B_{12}, A and B in complex preparation. For some time the patient was also given oral folic acid, 5 mg three times daily

After discharge the patient was seen in our Out patient Clinic every fourth week. There was no definite effect of the dietetic treatment, and as the patient ascribed some of his abdominal discomforts to it, the dietary regimen was abandoned. He began to complain of paresthesias of both lower limbs, and owing to increasing fatigue he was then readmitted in 1969 for further malabsorption studies which revealed severe steatorrhoea and pathological values of the Schilling, xylose and FIGLU tests. Free acid was present in the stomach There was megaloblastic dysplasia in the granulopoiesis (giant metamyelocytes, giant stab cells and macropolycytes characteristic of maturation disturbances I deficiency of antimegaloblastic factors (4)) and the serum folate concentration was low A the folic acid absorption (TRIFA) test (9) yielded low pathological values the administration of folic acid a resumed X-rays of the stomach and small intestine showed increasing dilatation of the duodenum and the upper portion of the jejunum, but the jejunal motility was apparently normal.

After discharge the patient became increasingly depressed by his condition and failed to return for heck ups for nearly 1 months, during which time he did not receive any substitution therapy On account of increasing numbness and asynergia of the legs he returned in 1970 and was gain admitted for renewed studies. He was now greatly emaciated and in poor general health Neurological xamination revealed pronounced ataxia, decreased deep and surface sensibility and spastic paresis of both lower limbs. H could walk with two forearm crutches, but wa absolutely helples in the dark.

The biochemical status was largely nchanged from the previous admi sion Gastrocamera examination was technically successful (1) howing normal stomach. As before, X-rays of the small intestine showed dilatation of the duodenum and the pper portion of the jejunum. I addition, several xposures (Fig) aroused suspicion of stenoses of the jejunum about 1 m distal to the Treitz ligament. Fluoroscopy revealed fluid su faces in the dilated jejunal loops, but the peristalsis preserved.

Owing to the continued progression of the patient disease—in particular the neurological defects—it was decided to perform exploratory laparotomy with view to relieving mechanical obstruction if any The operation revealed an intestinal tenosi tuated about 74 cm distal t the Treitz ligament, followed by dilated segment and then by another stenosis (Fig 3) Proximal to the stenosed segments the jejunum and duodenum were markedly dilated The intestinal wall as hyper trophied, and the segment howed igorous peristalsis. The stenosed portions of the intestine 35 cm in length, were resected, and an end-to-end anastomosis between the normal-appearing jejunum and the dilated proximal intestinal segment was carried out The postoperative course was uneventful Histological xamination of the resected specimen showed sequelae of an unspecific inflammation of the stenosed portions while the rest of the intestinal tissue was normal, part from hyper trophy of the lamina muscularis.

The substitution therapy was continued unchanged, and 6 months after the operation the patient was readmitted for malabsorption studies. The Hb content was 15 1 g/100 ml with 4.34 10^6 erythrocytes/µl, the peripheral blood and bone marrow were normal. The Schilling test with and without intrinsic factor the FIGLU TRIFA and xylose test showed normal values A moderate steatorrhoea of 7 g/24 hours per sisted X-rays of the small intestine showed les dilatation than on the previous occasions; the intestinal motility was normal.

Some neurological defects still but they



Fig 3 The strictured jejunum with dilated proximal segment.

seemed to be slowly regressi g. One year after the operation the patient general wellbeing was improved so much that he could be restored to his work as a photographer

DISCUSSION

The patient described suffered from malabsorption megaloblastic anaemia and neurological defects gradually resulting in severe disability He had for a long time had dyspeptic discomfort hich could be ascribed to the two strictures of he upper jejunum Apart from the irreparable neurological lesions all symptoms as well as the biochemical and physiological disturbances were relieved on reestablishing the intestinal passage by removal of the stenosed segment. The malabsorption syndrome may therefore be assumed to be a consequence of the intestinal stenosis and dilatation

It is not possible to offer an aetiological explanation of the stenosing process In some of the previously reported cases the strictures have undoubtedly been of tuberculous origin but in many of them the aetiology is unknown (3) Some authors have expressed the view that the strictures were an atypical variant of Crohn s disease (2). In the present case there was fibrosis referable to an unspecific inflammation but no granulomatous processes It is uncertain whether the inflammatory changes were of a primary or secondary nature

The megaloblastic anaemia must undoubtedly be ascribed to folate deficiency manifested by low folate concentrations in the serum with a pathologically high excretion of FIGLU substance in the urine Additional folic acid was given only intermittently and as the body depots contain folate sufficient for only 4 months consumption (13) It must be assumed that the patient had suffered from a severe folate deficiency The results of the TRIFA test show that the folate deficiency was due to reduced absorption of folic acid from the intestine The cause of this malabsorption is unknown As in the presence of obstructive intestinal lesions the bacterial flora often changes in the direction of the milieu present in the colon (11) it is reasonable to assume that the pH of the stagnant intestinal contents was changed in the alkaline direction If so the absorption of folic acid had been poor as it principally occurs at pH 6 (8)

As assessed by the Schilling test the intestinal absorption of vitamin B_{12} was also reduced It is assumed that intestinal stenosis with stagnation of the contents may lead to decreased absorption of intrinsic factor-bound vitamin B_{12} because of the presence of an abnormal vitamin B_{12}-consuming intestinal flora (16) Even though the intestinal microflora was not studied in the present patient the malabsorption of vitamin B_{12} demonstrated may very well be due to such a mechanism

The neurological defects ushered in by acro-

paresthesias and ending in incapacitating ataxia, are very similar to those of the subacute combined degeneration of the spinal cord which is seen in vitamin B_{12} deficiency Repeated determinations showed in fact decreased absorption of vitamin B_{12}, and as the neurological lesions principally developed during the period in which the patient failed to return for treatment with vitamin B_{12} it would be tempting to explain the cord degeneration as being due to lack of this vitamin. However against this assumption there is the fact that subnormal serum levels of vitamin B_{12} were not demonstrated at any time during the course of the disease The neurological lesions can therefore scarcely be ascribed to vitamin B_{12} deficiency On the other hand severe folate deficiency was demonstrated on several occasions and in spite of oral administration of folic acid low serum concentrations of folate were found which is in agreement with the assumption of a reduced intestinal absorption of folic acid

In general, folate deficiency is not assumed to result in neurological defects but nevertheless Reynolds et al. (15) often found folate deficiency in a series of patients with neurological disorders However it must be presumed that the state of malabsorption has comprised not only vitamin B_{12} and folic acid, but also other nutritional factors among which the lack of thiamine and niacin may have contributed to the development of the neurological disturbances None of these vitamins were given in the period during which the neurological manifestations showed the most rapid progression. In addition, the ataxia had been preceded by a pronounced weight loss It is therefore most probable that the subacute combined degeneration of the spinal cord was due to a deficiency of such nutritional factors as essential amino acids and certain members of the vitamin B complex However by reducing the absorptive capacity of the intestine for these factors (7) folate deficiency may indirectly have contributed to the development of the neurological disturbances.

The malabsorption syndrome associated with multiple strictures of the jejunum carries a grave prognosis and may in spite of optimal substitution therapy with known vitamins lead to severe disability of the patient. The treatment of choice is therefore as in the present case surgical relief of the jejunal strictures by which the intestinal malabsorption is to overcome

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OSLER S DISEASE WITH IMPAIRED ADHESION AND AGGREGATION OF PLATELETS

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Abstract In the investigation of haemostasis in 37 year-old woman with Osler s disease the Ivy bleeding time proved to be prolonged. Platelet adhesiveness, measured both by Hellem and Salzman methods, was decreased and platelet aggregation was clearly reduced after addition of ADP but only slightly after collagen and epinephrine. Platelet factor 3 activity was normal, as was clot retraction. The therapeutic consequences of the platelet defect, including the risk of using drugs affecting platelet function are stressed. It is concluded that in cases of Osler s disease the possibility of functional disorders of platelets should be borne in mind.

Vascular malformations are sometimes accompanied by a systemic disorder of haemostasis with a latent or manifest haemorrhagic diathesis as a result. Such a disorder has been demonstrated in haemangioma (7 12 13 14 15 19 1 23). It may also occur in Osler s disease or hereditary haemorrhagic telangiectasia (17) The author has observed a case of this disease with impaired adhesion and aggregation of the platelets The special therapeutic consequences that such a platelet defect might have were considered to justify a report of the case

CASE REPORT

The patient was a 37-year-old Jugoslavian woman who had been living in Sweden for 6 years. No skin or mucosal lesions like the patient nor bleeding symptoms were known in the family Two normal deliveries in 1958 and 1967 During the first pregnancy when the patient was living in Jugoslavia, she had had thrombophlebitis of the left lower leg. In 1966 she had had miscarriage. Menstruation had always been normal.

In 1969 the patient had painful bluish swelling of the tip of the right forefinger She sought medical advice and an incision resulted in profuse bleeding that required suturing of the kin Two years later she noticed bluish swelling in the proximal part of the right forefinger and in the right thumb wrist and l bow

Two days before admission to hospital in Oct 1971 she had found it difficult to open her mouth and to swallow At the same time she noticed an increasing swelling in the right half of the floor of the oral cavity behind the mandibular angle and along the lower jaw Neither then nor earlier had she ever had bleeding from the nose gastrointestinal tract or lungs.

On admission to the Department of Internal Diseases physical examination disclosed that the patient was in good general condition without cyanosis or dyspnoea. The lower legs showed bilateral varices Examination of the superficial lymph nodes, breasts, heart, lungs, abdomen and reflexes revealed nothing remarkable. The thyroid was moderately and diffusely enlarged BP 140/80

The oral and pharyngeal mucosa exhibited numerous red, scattered pin-point changes and, especially in the right buccal mucosa, a number of bluish, nodular lesions about 2×2–4×4 mm. Lesions of the latter type were also seen on the lower lip, the abdomen, on the distal part of the right forefinger and of somewhat larger size also over the right wrist and the right elbow In the right part of the floor of the oral cavity there was tangerine-sized fluctuant filling.

Routine laboratory investigations showed normal picture of the peripheral blood without anaemia or iron deficiency and a normal acid-base status. The oxygen saturation of Hb was 92–94%. Liver function studies disclosed normal values for serum enzymes and serum bilirubin. No proteinuria and normal sediment of freshly voided urine. ECG was normal. Chest X-ray showed an excavation about 2 cm long, possibly pressure erosion on the dorsal side of the left fourth rib.

Table I *Patterns of the coagulation and fibrinolytic systems*

	1st exami nation	2nd exami nation	Normal range
Coagulation time (min)			
Glass	8		6–14
Plastic	19		1 –32
Recalcification time (sec)	158		11 195
Factor VIII (%)	77		60–160
Factor IX (%)	98		60–160
One-stage prothrombin time (sec)	17 0		13–17
P&P (prothrombin+factors VII and X) (%)	87		80–120
Factor V (%)	72		80–120
Fibrinogen (g/100 ml)	0.26		0.20–0.40
Fibrinolytic activity on unheated fibrin plates			
Plasma (mm²)	23	0	0–50
Resusp euglob prec (mm²)	56	29	0–70
Euglobulin clot lysis time (min)	120	300	100–300
Plasminogen (%)	91		70–130
Inhibitors of plasminogen activation (%)		113	60–140
FDP (μg/ml)	25	15	0–5
Ethanol gelation test		Neg.	Neg.

COAGULATION STUDIES

Methods

The bleeding time was determined both by Duke's technique and by the method of Ivy as modified by Nilsson et al. (16). Patelet counts were made by the method of Björkman (4). Platelet adhesiveness was measured with the glass bead retention test, both according to (11) and to Salzman (20). Prothrombin consump- was tested according to Biggs and MacFarlane (3) retraction was determined according to Hart and Conley (10) Platelet aggregation was measured with Born's aggregometer (5) in the way described by Cronberg (6) after addition of ADP epinephrine and collagen and the values were compared with those simultaneously obtained in a healthy volunteer Other methods used were those formerly described (16)

RESULTS

Coagulation and fibrinolytic systems (Table I) The coagulation factors appeared normal except for a slight reduction of factor V activity The fibrinolytic system showed normal fibrinolytic activity normal content of plasminogen and of inhibitors of plasminogen activation Fibrinogen degradation products (FDP) in serum appeared however on both occasions The ethanol gelation test was negative

Platelet function (Table II) The bleeding time was normal according to Duke's method but clearly prolonged according to Ivy's. The platelet count was slightly reduced at the first examination, but normal at the second. Platelet adhesiveness was decreased as measured by Hellem's method as well as by Salzman's. Determination of aggregation showed clearly reduced sensitivity to ADP but only slight reduction on addition of epinephrine and collagen. Clot retraction was normal as was the prothrombin consumption test.

DISCUSSION

Though the patient did not know of such changes in any of her relatives, the disease was probably Osler's disease or hereditary haemorrhagic telangiectasia. Neither her parents nor siblings had had bleeding symptoms. But vascular malformations of this type may not cause bleeding disorders until late in life or even not at all

It is well known that in Osler's disease vascular malformations occur not only in the skin and the mucosa, but also elsewhere (2, 8 22, and others). In the present case chest X-ray revealed an excavation of a rib which might have been due to pressure from an arteriovenous aneurysm Owing to the haemostatic disorder it was decided to refrain from angiography This disease may also be associated with liver cirrhosis (1 9 24) Labora-

Table II *Studies of the platelet function*

	1st exami-nation	2nd exami-nation	Normal range
Bleeding time (min)			
Duke	1	2	1–5
Ivy	25	22	6–1
Platelet number $\times 10^3/\mu l$	120	190	150–400
Platelet adhesiveness (%)			
Hellem	15	15.5	17–33
Salzman		13.5	20–60
Prothrombin consumption test (%)	6		0–30
Platelet aggregation (Born)			
ADP	Decreased		
Epinephrine	Slightly decreased		
Collagen	Slightly decreased		
Clot retraction	Normal		

tory studies in the present case revealed no signs of liver disease which does not, of course, exclude the possibility of cirrhosis.

In an earlier investigation Muckle, using Borchgrevink's in vivo technique, found platelet adhesiveness to be decreased in Osler's disease (17).

In the present case examination of the initial phase of haemostasis showed the Ivy bleeding time to be prolonged but the Duke bleeding time to be normal. Duke's method is less sensitive and a latent tendency to bleeding owing to disturbances of the initial phase of haemostasis is often not revealed by this technique. The prolongation of the bleeding time was not due to thrombocytopenia, as the platelet count was only slightly reduced at the first examination and normal at the second, but to a qualitative change in the platelets with decreased adhesiveness and aggregation. Examination of the aggregability of the platelets showed decreased sensitivity to ADP but an almost normal aggregation after addition of epinephrine and collagen. This means that the ability of the platelets to release ADP was practically normal, i.e. the so-called release reaction was relatively intact. This is also supported by the fact that both the clot retraction, and the accessibility to platelet factor 3 measured with the prothrombin consumption test, were normal.

In the coagulation phase factor V activity was slightly decreased, but otherwise the reaction in this phase was normal. The fibrinolytic system showed FDP in the serum but no signs of increased systemic fibrinolytic activity. FDP occur not only in primary fibrinolysis but also in fibrinolysis secondary to disseminated or local deposition of fibrin. A disseminated deposition of fibrin results in an increased consumption of platelets and coagulation factors with reduced values. In association with so-called reactive processes fibrinogen and factor VIII increase, for which reason in such a situation normal values for these factors may be compatible with an increased consumption. In the present case, however, there were no signs of a reactive process. The normal values found for factor VIII and fibrinogen were therefore most likely not a manifestation of a relative decrease due to increased consumption, despite a tendency to thrombocytopenia and decreased factor V activity at the first examination. The negative ethanol gelation test also argues against disseminated intravascular coagulation. The occurrence of FDP should therefore be regarded as an expression of local deposition of fibrin, presumably secondary to the bleedings.

It is obvious that disturbed platelet function considerably increases the risk of bleeding associated with vascular malformations. In the present case it is true that bleedings resulting in hypovolaemia or anaemia had not yet occurred, but the submucous bleeding in the floor of the oral cavity, if it had progressed further, might have resulted in a life-threatening condition. Several of our most common analgetics and antiphlogistics, such as acetylsalicylic acid, indomethacine, phenylbutazone, can reduce platelet aggregation by inhibiting the release reaction and thereby the release of ADP necessary for aggregation. In the present case the sensitivity to ADP was decreased, while the ability to release ADP was practically un-

affected If the release reaction had also been inhibited by administration of a moderate amount of for example acetylsalicylic acid the risk of further bleeding would have been considerable

In decreased platelet adhesion and aggregation especially in cases with haemangioma or telan giectasia, agents reducing platelet aggregation such as acetylsalicylic acid indomethacine and phenylbutazone are contraindicated This applies also to substances which influence platelet ad hesion such as dipyridamole and what is of still greater practical importance dextran with a mole cular weight of 70 000 (Macrodex®) In a situation with severe loss of blood and hypovolaemia other plasma expanders or fresh blood should be used Transfusion of platelet concentrates may also be considered if available

A platelet defect in Osler s disease is thus of both prognostic and therapeutic significance *Patients with this disease should therefore be ex amined also for such a defect.* Ivy s method for determining the bleeding time covers platelet ac tivity fairly well in the initial stage of haemostasis although occasionally cases with defective platelet function and normal Ivy time may occur In prac tice, however this examination alone is sufficient provided that it is performed lege artis and by ex perienced examiners The coagulation phase is apparently of less interest in these cases unless liver function is impaired In supplementary investigation of the haemostasis the fibrinolytic ac tivity should also be determined but further investigation of the fibrinolytic system is presumably not necessary

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Acta med scand. Vol 196 pp 137–139 1974

ARTERIAL HYPERTENSION UNILATERAL CONTRACTED KIDNEY AND SUBCLAVIAN STEAL SYNDROME

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Abstract A case report is published concerning ar terial hypertension, unilateral contracted kidney subclavian steal syndrome, constriction of the right common carotid artery and hemolytic crisis. The renin concentration in the peripheral blood was found to be markedly increased. Following unilateral nephrectomy the renin concentration decreased to normal levels in the blood, but the hypertension remained. It is concluded that the elevated renin concentration was not the cause of the hypertension.

Subclavian steal syndrome is the name of a vascular anomaly characterized by a constriction or oc clusion of the subclavian artery on the cardial side of the left vertebral artery The consequence of this abnormality is that the blood supply to the left arm is effected via the retrograde blood flow through the vertebral artery resulting in temporary cases of cerebral hypoxia with dizziness and coma. The aim of the present case report is a description of a ery rare combination of arterial hypertension unilateral contracted kidney with elevated concentration of renin in the serum, subclavian steal syndrome constriction of the right common carotid artery and hemolytic anemia.

CASE REPORT

A 64-year-old woman was admitted to hospital on account of serious abdominal pains, nausea and vomiting. When she developed jaundice and levated values of the liver function tests, the first diagnosi was an attack of gallstones. But later the Coomb test was found positive, and the Hb concentration decreased after admission Therefore it was concluded that the patient was suffering from hemolytic crisis (Table I).

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BP was measured with different results in both arms about 140/90 mmHg in the left and bout 190/100 in the right. An arterial hypertension had been diagnosed about two years previously and the patient had been treated with methyldopa, hydrochlorothiazide and guanethidine for several month I the ward she was treated effectively with hydrochlorothiazide and beta idine, but when the BP decreased she developed syncopes convulsions and coma. These attacks and the different BP measurements in the left and the right arm caused suspicion of a vascular anomaly localized to the aortic arch or the arterial branches from the arch.

Aortic arteriography was performed and showed smooth stenosis of the right common carotid artery localized to the branching point of the carotid artery The lumen was reduced to about half the normal diameter Furthermore, there was constriction of the left subclavian artery and a retrograde filling of the left vertebral artery from the basis of the skull to the left subclavian artery *In this way the conditions for subclavian steal syndrome were proved.*

The next problem wa why the patient suffered from arterial hypertension measured in the right arm. About three years previously pyelography had been per formed in another hospital and hypoplastic left kidney was found Therefore a new pyelography was per formed, but no pyelogram appeared on the left side. I relation to the above mentioned aortic arteriography renal angiography was performed which showed nor mal artery supply to the right kidney but total oc clusion of the left renal artery 1 cm from the aorta. The kidney was contracted, measuring 9×2 / cm and was supplied with blood from collateral arteries. There fore the most likely explanation of the arterial hyper tension was renovascular hypertension. The renin concentration was measured in the peripheral blood and was found enormously elevated (725 μ GU/ml, normal 6–60 μ GU/ml) This apparently was in agreement with renovascular hypertension. The measurements were performed in the Department of Clinical Physiology Copenhagen County Hospital, Glostrup.

Following these diagnostic procedures the treatment of the patient was discussed with the vascular surgeons. It was decided to perform firstly plastic operation on the right common carotid artery and the left

Table 1 *Hematologic values during the hemolytic crisis*
Normal values given within parentheses

	Date of examination			
	22.11 71	25 11 71	11 12.71	15 12.71
Hb (g/100 ml) (11 7–15 7)	16.[illegible]	13 0	9.8	11.8
Hct (37–43)	49			35
Reticulocytes (<1%)				1.8
ESR (mm/h) (2 10)	87	120	95	102
Serum bilirubin (mg/100 ml) (0 2 1 2)	5.5	0 5	1.5	1 0
Serum haptoglobin (g/l) (0 60–3.2)	2.6			2.5
LDH (U/l) (100–370)	775			
Serum IgG (g/l) (7 4–14 2)	18.5			20 0
Serum creatinine (mg/100 ml) (0.8–1.2)	1 4	1 5	5.3	3.2

10 12 71 the serum contained markedly increased amounts of free autoantibody which reacted with enzyme technique. It was concluded that the patient suffered from an autoimmune hemolytic anemia and the direct Coombs test was positive.

clavian artery and secondly a left nephrectomy These operations were successf l and the patient is now free from the symptoms which led to admission to hospital and has no syncope and no convulsions. The renin concentration in the serum was normal after the nephrectomy (24 μ GU/ml) and the blood pressure decreased to normal levels (160/80 mmHg). Five months after the nephrectomy however it was again high 210/110 mmHg in the right arm and 190/110 mmHg in the left.

DISCUSSION

The patient history described is interesting with to the following points

Hemolytic crisis at the time of admission to ital 2) Subclavian steal syndrome 3) Constriction of the right common carotid artery 4) Arterial hypertension 5) Unilateral contracted kidney with elevated levels of *renin in the peripheral* blood 6) Unchanged arterial hypertension in spite of nephrectomy of the contracted kidney and normal level of renin concentration in the peripheral blood after the nephrectomy

1 The hemolytic crisis The hemolysis was Coombs -positive The cause of the hemolysis is still uncertain but it is well known that prolonged *treatment with methyldopa may provoke a* Coombs -positive hemolysis This patient had been treated with *methyldopa for two years*

2 and 3 The anatomical defects which are described *in cases of subclavian steal syndrome* were found in this patient but they were complicated by a constriction of the *right* common carotid artery These two bilateral constrictions may explain the dramatic result of the effective antihypertensive treatment, because it led to an insufficiency of the blood flow to the two cerebral hemispheres with syncopes coma and neurological symptoms like those seen in cases of cerebral hemorrhage. The arterial hypertension was an essential condition for a normal cerebral function.

4 5 and 6 The contracted left kidney with total occlusion of the renal artery was thought to be the cause of the arterial hypertension and this view was supported by the elevated concentration of renin in the peripheral blood In cases of renovascular hypertension the renin-angiotensin system has been considered as the causal factor but no evidence exists for this view at the present time (1 2 4 6) In the present case the renin concentration decreased to normal levels in the *peripheral blood after the left nephrectomy* but in spite of this the BP increased after the operation to almost preoperative levels Therefore we may conclude that in our patient the renin-angiotensin system was not responsible for the arterial hypertension. Maybe the elevated level of renin *in the peripheral blood was only a symptom of a* destructive process in the left kidney with a *liberation of renin to the peripheral blood* That enzymes are liberated to the peripheral blood in *cases of organ-destructive processes* is well known It may take place in e.g. myocardial infarction and liver diseases

The etiology of the multiple stenoses of the arteries was not clarified because only the removed left kidney was studied under the micro-

scope but not the constricted parts of the carotid and the subclavian arteries. The renal artery was constricted on account of endarteritis obliterans. Other possible causes of constriction of arteries besides atherosclerosis are polymyositis arteritica (5) or fibromuscular hyperplasia.

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HYPERTENSIVE CRISIS FOLLOWED BY ADRENOCORTICAL INSUFFICIENCY AFTER UNILATERAL ADRENAL PHLEBOGRAPHY IN A PATIENT WITH CUSHING S SYNDROME

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Abstract A report is given of patient with Cushing s syndrome who developed serious hypertensive crisis later followed by adrenocortical insufficiency after retrograde adrenal venography It is concluded that this examination should be done only in selected patients ho have to be kept under caref l observation for hours following the procedure.

In both Cushing s syndrome and primary aldosteronism the preoperative differentiation between an adrenocortical tumour and a bilateral hyper plasia is important. The diagnosis of adrenocortical hyperfunction rests upon biochemical criteria. Demonstration of an adrenocortical tumour confirms the biochemical diagnosis and gives the side-localization. This makes selective surgery possible as opposed to bilateral adrenal exploration. A relatively large tumour seldom escapes detection by either retroperitoneal pneumography or by arteriography The smaller ade nomas, most often found n patients with primary aldosteronism, will however rarely be detected by these methods. In Conn s syndrome the dif ferentiation between bilateral hyperplasia and uni lateral tumour is of special importance as patients with bilateral hyperplasia, present in 14–30% of cases (2, 4), seldom benefit from adrenalectomy and should therefore not be subjected to surgical treatment (2). Unlike the case with Cushing s syndrome, biochemical differentiation between hyperplasia and tumour is not possible in patients with primary aldosteronism

By means of retrograde adrenal phlebography (1 10, 12), it is however possible to detect even small adrenocortical adenomas Besides visualiza tion of the adrenal gland the method enables a comparison to be made between the concentrations of adrenocortical hormones in blood specimens taken from each of the two adrenal veins and from vena cava inferior (10 15) Although it may be technically difficult to perform selective catheter ization of the adrenal veins, especially the right one a comparison of blood concentrations of corticosteroids in one adrenal vein with that of vena cava inferior distal to the renal veins may give information of value for the differentiation of a unilateral tumour and a bilateral process (15)

Selective retrograde adrenal phlebography is however not without risks to the patient as is demonstrated by the following case report

CASE REPORT

The patient, 64-year-old woman, was admitted to our department in 1972 In the last 3 years she had been treated with diet for mild diabetes mellitus. Her main complaint was intense backache and difficulties in walking due to an extreme osteoporosis of the spine with crush fractures of multiple vertebrae. Her BP was not elevated. She had not typical moonface but there was marked growth of facial lanugo hairs The skin was thin and atrophic with subcutaneous haemor rhages but without striae. The prominent abdomen contrasted with her thin extremities, which showed muscular atrophy

The clinical suspicion of Cushing syndrome was confirmed by biochemical studies. Although the morning values for plasma cortisol concentration were not elevated, the diurnal variation was absent. Plasma cortisol determined fluorometrically (9) was at 8 a.m. within the range of 19.0–22.0 μg/100 ml, at 8 p.m. 17.8–19 8 μg/100 ml. The urinary excretion of 17 OHCS (11) wa 19 9–28.8 mg/24 h (normal values for age and se 2 0–10 0). The rinary excretion of 17-KS was only moderately increased

Neither the plasma cortisol concentration at 8 a.m. nor the urinary excretion of 17-OHCS was suppressed to values lower than 16.8 μg/100 ml and 18.5 mg/24 h respectively even after dexamethasone 2 mg every 6 hours for 2 days. The plasma cortisol concentration increased sevenfold after ACTH administration while there was no response to metyrapone. Retroperitoneal pneumography performed to visualize the adrenals was inconclusive. Due to technical difficulties selective catheterization of the right adrenal vein was not successful. Blood samples from the left adrenal vein contained a high concentration of cortisol (approximately 900 μg/100 ml). A blood specimen taken from vena cava inferior also contained high plasma cortisol concentration (approximately 300 μg/100 ml) while the concentration in a cubital vein was 20.2 μg/100 ml. With the catheter in the left adrenal vein about 10 ml of the contrast medium, Isopaque® (Nyco) was hand-injected with increased rapidity until the patient felt slight discomfort in her left flank. The phlebography showed an enlarged left adrenal gland containing no visible tumour.

A few hours after the examination the patient complained of increasing pain in her left flank, with tenderness on palpation. Gradually a state of shock developed with anuria and mental confusion. Although her skin became cool moist and pale the BP was found unexpectedly to have risen from 120/80 to 10/1.5 mmHg, and the pulse was strong and rapid. Lowering the BP with α-blocking agents (phentolamine and chlorpromazine) brought the patient out of shock, but repeated administrations were necessary for the first 12 hours.

A urinary specimen sampled in the next morning indicated a markedly increased excretion of catecholamine metabolites. The excretion of vanyl-glycolic acid as 35 μg/mg creatinine (normal 1.5–4.0) which ion increased ver the next 5 days.

Although the patient improved initially weakness and ... persisted with a BP below 100/60 mmHg. On the third day after phlebography she became critically ill but impro ed promptly after i administration of hydrocortisone. Unfortunately plasma for determination of corticosteroids wa not sampled before treatment. After five weeks oral treatment the corticosteroids were withdrawn. The plasma cortisol concentration at 8 a.m. in the next morning was 2.7 μg/100 ml and did not increase after ACTH (Synacthen, Depot® Ciba, 1 mg i.m. for 3 days). The plasma aldosterone concentration (16) was also low 6.0 pg/ml (normal 25–150) and unresponsive to ACTH.

The treatment with corticosteroids had to be resumed. Repeated attempts to withdraw the treatment have been without success, the patient still needs cortisone 25 mg/day. Half a year after the adrenal phlebography plasma cortisol determined by competitive protein-binding assay (13) rose from ..7 to 1.0 μg/100 ml after repeated injection of ACTH (Synacthen Depot). The urinary excretion of 17-OHCS rose from ..2 to 23.8 mg/24 h. The patient was treated with dexamethasone during the test.

COMMENT

The patient was a neglected case of Cushing's syndrome presenting with only a few of the typical clinical signs of the disease. Biochemically the probable pathoanatomical diagnosis was an adrenocortical adenoma while hyperplasia was the most likely radiological diagnosis. The high plasma cortisol concentration in the left adrenal vein indicated an increased cortisol production at this side. The high value found in "the inferior vena cava" probably means that this specimen had been sampled from the left renal vein into which the left adrenal vein empties and therefore was of no diagnostic value.

For this patient the differentiation between an adrenocortical tumour and bilateral hyperplasia was most important. A tumour would have had to be removed surgically but if the Cushing's syndrome was due to hyperplasia in this fragile patient we should first have tried pituitary irradiation instead of performing the usual bilateral adrenalectomy.

As it was the adrenal phlebography performed for diagnostic purposes turned out to be "therapeutic". Eagan and Page (5) described a patient who developed adrenal insufficiency following bilateral adrenal venography. A cure of Cushing's syndrome after an apparent destruction of an adrenal adenoma following unilateral adrenal phlebography has also been reported (6). The same applies to a patient with primary aldosteronism (7). The mechanism is thought to be a haemorrhagic destruction of the adrenal cortex or of the cortical tumour due to the increased fragility of the veins in these patients. Bookstein et al. (3) report on the frequent occurrence of intraadrenal haemorrhage after adrenal phlebography. Although finding the method safe and reliable in other patients they consider the risk of serious complications to be so high that they do not recommend this examination as a routine in patients with adrenocortical hyperfunction.

In our patient the primary complicating mechanism has presumably also been an intraadrenal haemorrhage. Judging both from the clinical symptoms and from the observation of an excessively high excretion of catecholamine metabolites in the urine the haemorrhagic lesion must have initially affected the adrenal marrow. It is also possible that the adrenal cortex has been directly

affected by the intraadrenal haemorrhage. To explain the subsequent adrenal insufficiency we then must presume the left adrenal gland to have harboured an autonomous tumour suppressing the opposite gland.

Another possible mechanism is adrenocortical arteriolar constriction with bilateral adrenocortical necrosis due to the catecholamine efflux. Gold et al. (8) have reported on a hypertensive crisis following adrenal venography in a patient with phaeochromocytoma. Hypertensive episodes due to adrenal phlebography have also occurred in patients with normal adrenal glands (12, 14) but in no instance known before has this mechanism been found responsible for the later development of adrenocortical insufficiency.

Because of the hazard of both a hypertensive catecholamine crisis and the risk of inducing adrenocortical insufficiency, adrenal phlebography should only be performed in selected cases. The pressure and volume of dye injection should be controlled carefully and the patient kept under close observation for several hours following the examination. Development of a hypertensive crisis should be promptly treated with α-blocking *agents*.

Bilateral catheterization of the adrenal veins with sampling of blood for corticosteroid analyses but without contrast injections will most often be safe and informative procedure. Thrombosis of the iliac and of the adrenal veins, however, has been reported (1, 12).

At present there appears to be less need for the method of adrenal phlebography as adrenal photoscanning following injection of ^{131}J-cholesterol may accurately differentiate between unilateral and bilateral adrenocortical hyperfunction (4, 17) and this method involves no risk to the patient.

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Announcements

The annual Ultrasound Conference will be held in Seattle Washington USA Oct. 5–10 1974 The American Society of Ultrasound Technical Specialists (ASUTS) will hold their meeting on Oct 5 the Basic Education and Advanced Education sessions will be held on Oct. 6–7 and the American Institute of Ultrasound in Medicine (AIUM) scientific sessions on Oct. 8–10

A postgraduate education course entitled Diseases of the liver will be presented by the Department of Medicine University of Miami School of Medicine Miami, Florida Nov 21–23 1974

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Again this year I am compiling case reports of allergic reactions to biting insects i.e. mosquitoes fleas gnats kissing bugs bedbugs chiggers, black flies horseflies sandflies deerflies etc I am also interested in reactions to the Imported and Southern Fire Ants.

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EDITORIAL

RENAISSANCE OF A NEW FRONTIER IN OBESITY RESEARCH

All who have treated severe obesity by conventional therapeutic measures aiming at a negative caloric balance know how extremely difficult this is. This is also massively documented in the literature. The successful treatment of a patient with severe obesity defined as a maintained body weight within the limits of approximately normal is probably seen very seldom After having tried to treat several hundred such patients the experience of the author is that when severe hyperplastic obese patients continue to reduce weight on an outpatient basis and approach the normal body weight, then there is some additional factor contributing to weight decrease and occult malignant disease should be remembered

Why then, is severe obesity so difficult to treat in practice when the theory seems so easy? Are there factors which we still do not know sufficiently well and if so which may such factors be?

Energy balance

The first law of thermodynamics does of course apply also to severely obese subjects Why then, do they not decrease to normal body weight in spite of high motivation and hard trying This must be because they are not in a negative caloric balance signifying that caloric intake is sufficiently reduced in relation to caloric output or that the caloric output is smaller than anticipated Usually in practice the main emphasis is placed on the caloric intake side the patient being more or less openly suspected to have a hidden caloric intake. This side of the equation is however easy to check there are precise methods to determine the caloric equivalents of dietary intake and to assure that these calories have indeed been removed from the gastrointestinal tract.

The first factor on the caloric output side of the equation is of course physical activity Obese subjects have been found often to be physically inactive. There is however also another factor which is not directly associated with physical activity and which might cause a variation in caloric output. This is the caloric expense for other purposes than muscle work, and here the methodological difficulties are much greater than when caloric intake is measured Particularly for long-time purposes these methods are very impracticable. This fact alone implies that it might be worthwhile to search for unknown factors which may explain the enigma of the failure of treatment of severe obesity

Technical errors in measuring caloric output

Let us take some striking examples of the lack of precision of the methods applied for measurements of the caloric output due to other factors than physical activity The accepted normal variation of $\pm 10\%$ in basal metabolic rate allows variation of oxygen uptake in absolute terms which may differ between comparable individuals by a factor of 2. This methodological error could account for several kg fat per year

Another more genuine methodological difficulty is to estimate the caloric contents remaining in the body factor necessary to know when evaluating caloric balance One kg adipose tissue contains at least ten times more calories than one kg lean tissue. Furthermore the caloric content of water is zero a fact which frequently seems to be forgotten in obesity treatment practice Clearly then, body weight is not a sufficient measure one needs to know the exact amounts of body fat body water and lean body weight. The possibility of obtaining exact measurements seems best for body water but is considerably less for body fat A shift of weight of several kg from lean tissue to fat tissue corresponding to several tens of thousands of calories might well escape notice with the presently available techniques This means that

experiments with energy balance measurements have to be performed over sufficiently long periods. Instead, unfortunately difficulties in measurement of the lost calories then appear To measure heat production or oxygen uptake accurately over long periods seems in fact impossible with available techniques.

These methodological difficulties have probably prevented meaningful conclusions within this field. The clinical problem of obesity does not become smaller through the fact that weight increase is often a very slow process In order to trace the reason for a weight increase of 5 kg per year one needs to be able to measure differences of the order of 100 calories per day This is probably beyond the capacity of our presently available methods.

The Vermont study

Recent studies by a group in Vermont, USA (6) have in a most dramatic way brought this question into focus These studies are impressive because the time axis is so long that the methodological problems mentioned are probably of minor importance These investigators overfed young men *with more than 5 000 calories per day for several* months causing an excess of more than 1 mill calories in some cases In spite of this magnificent overfeeding under controlled conditions some of the volunteers did not increase in body weight. This might be the reverse situation to the unsuccessful unhappy dieting patient, who does not reduce weight in spite of reduction of caloric intake. This points clearly to the fact that there is yet much to learn about energy regulation and within this field of ignorance the explanation of the enigma of the unsuccessful dieting patient may well be hidden Let us therefore look into some more arguments within this field of obesity research which has in fact attracted the interest of several recent international congresses

Old and recent information on caloric expenditure

The phenomenon of the subject who can eat *whatever be wants without increasing in weight is* well known. This empirical fact was recorded at the beginning of this century by Neumann (5) in studies on himself Neumann noted that he could eat a wide range of calories but his body weight remained constant. *It could be argued that he did* not know his body composition before and after the dietary periods. A shift in lean and fat body mass could explain missing calories Furthermore his physical activity was not know This is true but the observation periods were so long that these errors probably cancel out.

These were the first attempts to document the empirical observations The *Vermont study (6)* and other studies referred to below then constitute a renaissance of an old frontier in obesity research

Miller (3) reviewed some of these aspects in a recent congress in the following way Careful *studies of the caloric intake in a given population* at a given body weight reveal that there is indeed a large variation Actually some adults seem to manage to keep their body weight constant on the same caloric consumption as babies The implication is according to Miller that caloric intake has comparatively little to do with the resulting body weight.

Another example taken by Miller to demonstrate the potential possibilities within this field is the variation of basal metabolic rate mentioned above. Obviously when the method is so crude large differences could easily be hidden during long periods Nevertheless careful measurements of *absolute oxygen uptake rates in different experi*mental situations have revealed small differences which when multiplied by the time factor become most important

Basal metabolic rate is the oxygen uptake after sleep in the fasting and in resting state Possible differences between different situations of interest are most likely minimized by these ambitions to standardize the situation examined It seems more logical to perform the measurements after a standardized work load in the non-fasting state Then the caloric expenditure is increased and potential differences between situations of interest should be accentuated When performing these experiments Miller et al (4) found that the energy output (oxygen uptake measurements) increases in subjects fed a hypercaloric diet, which might explain the lack of increase in body weight in some of these subjects When measuring oxygen uptake *over whole days* it seems possible to explain satisfactorily the extra output of calories

Is there a regulation of caloric expenditure

It is obvious that within this field very small differences in caloric intake and output play a large role over a year or more in terms of kg body fat.

It is therefore surprising that the caloric intake of different weight-constant subjects differs so vastly also under strictly controlled conditions during long periods. This leads Miller and others to believe that the regulation of body weight and body fat resides mainly on the caloric output side the caloric intake playing a comparatively minor role. The output regulation is mainly due to the efficiency of handling calories directing them to heat or to body fat.

Careful measurements have thus given experimental support to the idea that, in fact, there is probably a regulatory mechanism on the caloric output side. The next question is then how this can be explained on the biochemical and/or perhaps endocrine basis Several theoretical explanations remain. These could be such phenomena as uncoupling of oxidative phosphorylation, or more specific energy leaking pathways of carbohydrate protein and lipid metabolism None has so far been convincingly demonstrated, but here too the possibilities of exact evaluation are limited.

This of course leaves us with the potential possibility that obesity is caused not so much by over eating but by a deficient system for disposing extra calories. Luxus Konsumtion a term coined by the early German workers signifying the potential to get rid of extra luxus" calories is not at work in subjects tending to become obese They become obese by accumulating more ingested calories than do subjects without tendency to obesity From this point of view accordingly it is normal to dispose of some ingested calories and abnormal to be too efficient in saving calories

Adipose tissue cellularity and nergy expenditure

The recent development of the cellular concept of adipose tissue and its relation to obesity seems to have a potential to create a better understanding of obesity These observations may help mainly to distinguish different subgroups of obesity and therefore different possibilities for among other things, pathogenesis The hyperplastic obese patients are those who are most difficult to reduce to a normal body weight by introduction of a negative caloric balance (1). Furthermore when subjected to physical training, their weight reduction is abnormally slow ( ) Weight decrease seems to cease at a certain fat cell size and seems actually possible to predict approximately when fat cell size is known at the start of treatment (1). These observations imply that there may be some association between ad pose tissue cellularity and the possible caloric expenditure regulation.

The new information briefly reviewed here is still fairly preliminary in spite of the fact that the observations are old both empirically and in research Nevertheless, it may open up means to solve a dilemma which most obesity treating doctors not to mention the poor obese patient have experienced as most frustrating namely why some people increase in weight while others do not and why is conventional treatment of obesity very seldom successful

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BOOK REVIEW

Minimal brain dysfunction. New York Academy of Sciences Vol 205 Edited by Felix F de la Cruz, Bernhard H. Fox and Richard H. Roberts. 396 pages. $30. New York 1973

Children with obvious handicaps, e.g. motor disabilities such as cerebral palsy syndromes, or intellectual defects such as mental retardation have successively been accepted as important groups for active habilitation programmes. Until recently probably much larger numbers of children with very slight deviations of different cerebral function have not been recognized more often been misconceived as "difficult or "naughty" and have not received the necessary understanding and help in daily life and school Some of these children have later in life been labelled as "lesional syndromes" by child psychiatrists and as minimal cerebral palsy" or "minimal brain dysfunction" (MBD) by neuropediatricians. These children are mainly characterized by slightly retarded psychomotor development, defective servocontrol of their motor function, impaired body image often very incapacitating perceptual difficulties, concentration disturbances with short attention span, emotional lability and hyperirritability and inability for abstract thought—but they have no or only very soft neurological abnormalities of conventional type

This volume from NYAS collects a considerable amount of recent knowledge presented in different papers at a conference organized by the Academy in cooperation with the National Institute of Child Health and Human Development and the National Institute of Neurological Diseases and Stroke on March 20-22, 1972. Like all books of this type the level is uneven but by and large it gives a good insight into many of the multifaceted aspects of MBD For the referee papers of particular clinical interest were "Follow up of infants at risk of minor brain dysfunctions" by the Prechtl group from Groningen and with a very strict scientific approach and The natural life history of children with minimal brain dysfunction" by Dr Eric Denhoff from the Providence Center with his broad clinical experience of these problems over many years.

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ON A FAMILY WITH COEXISTENCE OF PHENOTYPES II AND III HYPERLIPOPROTEINEMIA

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Abstract Two brothers with xanthomata and coronary heart disease presented all the clinical and lipid-chemical characteristics of type III hyperlipoproteinemia. Preparative ultracentrifugation at density 1 006 revealed β-migrating lipoproteins in the supernatant fraction, and paper electrophoresis showed a "broad β band" Not only palpebral xanthelasmata and tendinous and tuberous xanthomata but also xanthochromia striata palmaris, so far only seen in types III and IV hyperlipoproteinemia, were present. However the family history indicated genetic type II, because 6 of the 8 sibs of the probands showed genuine type IIa hyperlipoproteinemia. The implications of these observations are discussed.

In 1965 Fredrickson et al. (5) described a method permitting better phenotyping of primary hyperlipoproteinemia on the basis of an improved paper electrophoretic separation of serum lipoproteins This system seemed to lead to a simple classification into five types but the authors recognized that in type III hyperlipoproteinemia two subtypes could be *distinguished* one characterized by increased amounts of Sf 12–20 lipoproteins and a broad band extending from the β to the pre-β position in paper electrophoresis the other showing increased amounts of Sf 0–12 of the low density lipoprotein (LDL) fraction in combination with an increased Sf 20–400 fraction resulting in separate β and pre-β bands (5) It is claimed that these problem cases can now be satisfactorily identified by a modified classification (2), based on combined paper electrophoretic and ultracentrifugal analysis as a type IIb and type III with as differential diagnostic criterion the presence or absence of "floating β lipoproteins" (4) whose presence is considered to be pathognomic of type III Substantial evidence has been presented that a family with hyperlipoproteinemia can have members with type IIa (the "pure" hypercholesterolemia) and others with type IIb (4 10).

The present paper described a family in which phenotypes II and III coexist, a situation which to the best of our knowledge has not been previously reported

PATIENTS

Case 1 A 48-year-old man, first seen in Sept. 1965 He had suffered from angina pectoris for 4 years, during which period multiple purplish-brown tumours had developed on his elbows and yellowish streaks in the creases of the palms of his hands. He had been on a low-fat diet with restriction of alcohol. No other anamnestic data are available

At examination he showed tuberous xanthomata on both elbows, xanthochromia striata palmaris (Fig. 1) prominent tibial tubera, and corneal arcus in each eye BP 115/75 mmHg, ECG and chest X-ray normal. Weight 77 kg, height 167 cm.

Case 2 Brother of case 1 aged 45 years, examined in March 1966. For 15 years he had had yellow plaques on his eyelids and lumps in the tendons on the back of his hands. He had suffered from angina pectoris since 1960 and in Dec. 1965 he had had myocardial infarction, after which he had been on anticoagulants and low-fat diet, with complete restriction of alcohol and smoking.

He showed palpebral xanthelasmata (Fig. 2), tendinous xanthomata on the extensor tendons of both hands (Fig. 3), xanthochromia striata palmaris, tuberous xanthoma on one arm, and corneal arcus in each eye. BP 120/80 mmHg, ECG showed definite signs of myocardial infarction, chest X-rays were normal. Weight 93.3 kg, height 182 cm.

The relevant laboratory data of both patients, including blood count, fasting blood sugar thymol turbidity alkaline phosphatase creatinine protein electrophoresis, urinalysis and 24-hour urinary excretion of 17 ketogens, were all within normal limits.



Fig 1 Xanthochromia striata palmaris in patient 1



Fig 3 Xanthomata tendinosum in patient 2.



Fig 2 Xanthelasmata palpebralis in patient 2.

The father of the patients, who had palpebral xanthelasmata, died suddenly at the age of 67 The mother died at 59 of cancer A younger sister was reported to have diabetes mellitus and hypercholesterolemia. Unfortunately she was out of reach for examination.

Both patients were married Each of them had four children, aged 6–26 years, all apparently in good health and completely normal on physical examination.

METHODS

Blood was collected after 12–24-hours fast, 22.5 ml whole blood being drawn into a 25 ml flask, containing 2.5 ml of standard citrate solution (33 g disodium citrate/l). For the preparation of serum a 10 ml blood sample was obtained by centrifugation for 5 min at 2 500 rpm to precipitate the blood cells and then for 20 min at 4 000 rpm to remove platelets. Serum was obtained by centrifugation of blood kept at 4°C for 4 hours. Lipoprotein fractions were obtained by combination of ultracentrifugation and precipitation (6, 13) The purified plasma and the serum lipoprotein fractions were extracted with methanol-chloroform mixtures. The non-

Table I *Lipid values*

TL=total lipid, TS=total cholesterol, PL=phospholipids FC=free cholesterol, CE=cholesterol esters, TG triglycerides, FFA=free fatty acids

	Pedigree no	Lipids (mg/100 cm³)							% of total lipids				
		TL	TS	PL	FC	CE	TG	FFA	PL	FC	CE	TG	FFA
Case 1	II 1	1 676	533	486	153	646	381	10	29	9	38	23	1
Wife	II 2	631	208	215	57	252	101	6	34	9	40	16	1
Child	III 1	1 070	399	366	109	494	82	19	34	10	46	8	2
Child	III 3	1 073	391	374	91	510	68	30	35	9	47	6	3
Child	III 4	996	377	342	94	482	41	37	34	10	48	4	4
Case 2	II 3	2 018	541	474	170	603	722	22	23	8	32	37	1
Wife	II 4	776	243	278	65	302	95	36	36	9	39	12	5
Child	III 5	1 354	518	428	153	619	118	36	31	11	46	9	3
Child	III 6	709	206	206	57	255	170	21	29	8	36	24	3
Child	III 7	1 061	405	325	107	507	95	28	30	10	48	9	3
Child	III 8	819	305	277	71	388	53	20	34	9	49	6	

Table II. *Lipid content of the various lipoprotei fractions*

CH=chylomicrons VLDI =very low density lipoproteins, LDL=low density lipoproteins, HDL=high density lipoproteins

	Pedigree no	Sex	Age (y)	Lipoprotein lipids (mg/100 ml) CH	VLDL	LDL	HDL	% of total lipids CH	VLDL	LDL	HDL
Case 1	II 1	♂	48	83	647	780	166	5	39	47	10
Wife	II 2	♀	47	6	95	334	196	1	15	53	31
Child	III 1	♀	23	3	54	845	130	3	5	79	13
Child	III 3	♀	18	21	53	859	140		5	80	13
Child	III 4	♀	13	30	20	757	189	3		76	19
Case 2	II 3	♂	45	L.I	1 009	787	101	6	50	39	5
Wife	II 4	♀	44	16	109	465	186	2	14	60	4
Child	III 5	♂	21	41	136	1 001	176	3	10	74	13
Child	III 6	♂	1	7	41	333	128	1	34	47	18
Child	III 7	♀	14	21	116	785	138	2	11	74	13
Child	III 8	♀	6	8	37	631	148	1	4	77	18

lipid material in the combined extracts was removed by Folch partition (3)

Determination of total lipids, phospholipids free and total cholesterol triglycerides, and free fatty acids were carried out as described elsewhere (13). Paper electrophoresis was performed according to the method of Lees and Hatch (8)

RESULTS

Both patients showed xanthochromia striata palmaris, which is a two-dimensional analogue and precursor of the xanthoma striata palmare (1) In the literature they are sometimes designated as planar xanthomata of the palms but this term is confusing (1)

Plasma lipid analysis (Table I) revealed in both

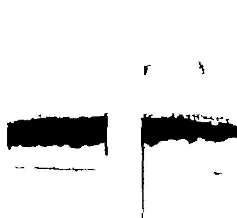


Fig 4 Broad β band on paper electrophoresis in patients 1 and 2.

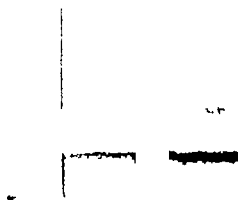


Fig 5 β moving VLDL ("floating β") on paper electrophoresis in patient 2. VLDL to the left, LDL+HDL to the right

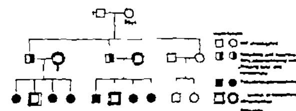


Fig. 6. Pedigree of the family.

brothers a combined hypercholesterolemia and hypertriglyceridemia with a cholesterol/triglyceride ratio of about 1.35. Paper electrophoresis showed a broad β band (Fig. 4). In the preparative ultracentrifuge (Table II) the LDL fraction (density 1.006–1.063) was moderately increased, the very low density lipoprotein (VLDL) plasma fraction (density <1.006) greatly increased. The plasma VLDL, isolated by preparative ultracentrifugation at density 1.006, migrated paper electrophoretically in the β region (specific for "floating β" lipoproteins) (Fig. 5).

Six of the 8 children presented a type IIa hyperlipoproteinemia (Fig. 6). Their serum lipid and lipoprotein values are given in Tables I and II. All 6 have increased LDL and normal VLDL concentrations, increased plasma cholesterol and a chol./TG ratio > . Floating βs were not found. Paper electrophoretically they all showed an in-...sed intensity of the β band and a normal pre β ...nd.

DISCUSSION

According to the WHO classification ( ) type IIb hyperlipoproteinemia is chemically characterized by an increase in both LDL and VLDL. On electrophoresis they show distinct β and pre β bands. Type III hyperlipoproteinemia should have one unequivocal criterion: the presence in the plasma of an abnormal VLDL with β electrophoretic mobility. Furthermore, paper electrophoresis usually shows a "broad β band" extending from the β to the pre-β position. Both of our probands fulfil these criteria for the diagnosis of type III hyperlipoproteinemia: floating β lipoproteins were demonstrated in their serum, and a "broad β band" was found on paper electrophoresis. The quantitative lipoprotein and plasma lipid analyses supported this diagnosis. Furthermore xanthochromia striata palmaris, so far only seen in type III and type IV patients (11), occurred in the palmar creases of these probands.

Our investigations show, however, that 6 of the 8 sibs of the probands are heterozygous type IIa. Consequently, both probands must be carriers of the genetic trait responsible for type II, both mothers of the IIa siblings being normal.

The following possibilities can now be considered. (*a*) All of the presented patients are genetically type II. In the probands the "floating β" may be an epiphenomenon, as is suggested by the fact that it was found only at the first examinations and disappeared during diet therapy. The children might also present phenotype IIb or even III later in life. (*b*) In this family two genetic traits coincide: the dominant type II and the incomplete dominant type III (4, 10), which is not yet expressed in the siblings. The xanthoma pattern in the probands favours this hypothesis. A conclusive choice between these possibilities cannot be made yet.

The present study, however, emphasizes that the relationship between genetic classification and lipoprotein phenotype is not a single and specific one. The occurrence of phenotypes III and IV in the same family has already been reported (4, 9). This study brings unequivocal evidence for the presence of phenotypes IIa and III in a single family. Also Hazzard et al. (7) concluded from a study of survivors of myocardial infarction that β VLDL may not be a specific marker for a single and distinct lipid disorder. Contrary to a previous assumption (2) it appears also from our data that the presence of β VLDL in a patient and a first degree relative may not be pathognomonic for a definite genetically determined type III lipoprotein disorder.

Note at the correction

We were finally able to examine the blood of child III 2, aged 26. The lipid and lipoprotein values proved to be within the normal range. Lipids: TL 851, TS 289, PL 272 (32 %), FC 68 (8 %), CE 383 (45 %), TG 26 (1 %), FFA 76 (3 %). Lipoproteins: CH 9 (1 %), VLDL 153 (18 %), LDL 44- (52 %), HDL 247 (29 %).

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SERUM LIPID AND LIPOPROTEIN CONCENTRATIONS IN A NORWEGIAN POPULATION SAMPLE

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Abstract Serum lipids and electrophoretically deter mined lipoprotein concentrations have been analysed in 168 healthy subjects (118 males and 50 females) belonging to two different age groups. An increase in serum lipid and lipoprotein levels with age was seen No dif ference was found between the concentrations in young males and females. Higher concentrations of β- and pre β-lipoproteins and lower level of α-lipoproteins were found in males than in females in the age group 40–59 Serum cholesterol values were determined in a series of 828 males and 382 females of different ages (15–69 years). In males the mean cholesterol values increased from 197 mg/100 ml in the age group 15–19 to 280 mg/100 ml in the age group 40–49 Means close to the lat ter age value were found also in the higher age groups. In females, mean cholesterol value increased throughout life from 208 mg/100 ml in young females to 337 mg/100 ml in those aged 60–69

There appears to be no general agreement on the "normal values for plasma lipids or lipoproteins For the purpose of establishing reference values for further genetic studies in hyperlipoproteinaemic subjects and their families lipid and lipoprotein analyses were conducted in several groups of per sons.

MATERIAL AND METHODS

Subjects Four groups (A–D) of healthy individuals were randomly selected and all subjects in these groups were interviewed and subjected to clinical examination Symptoms or signs of cardiovascular disease were not revealed in any of the subjects. N person used drugs known to influence lipid or lipoprotein levels. All blood samples were drawn after an at least 10-hour fast, and serum lipids and lipoproteins were studied.

Research fellow the Norwegian Council on Cardiovascular Diseases.

The composition of the four groups was as follows. group A. 25 males. 18–24 years old (mean 21) group B 25 females, 21–24 years old (mean 22), group C 93 males, 40–59 years old (mean 48), group D 25 females, 40–59 years old (mean 48).

In two additional groups (E and F) of 15–69 years old persons the concentration of serum cholesterol only was investigated. Group E consisted of 828 men and group F of 382 women, sampled at voluntary health control. Within each group the numbers of individuals with muscular and with sedentary work were approx imately equal Most, but not all subjects were in the fasting state when blood was collected N individuals were excluded from either of these two groups

S rum samples Blood was collected by venepuncture and allowed to clot for 2–4 hours, and the serum was then pipetted off Lipoprotein lectrophoresis was per formed within two days on small aliquots kept at 4°C, whereas the major part of the serum was kept at −28°C until the lipid analyses could be performed

On samples from series A–D simultaneous determinations of serum cholesterol and triglycerides ere car ried out in an AutoAnalyzer® by the methods of Block et al (2) and Kessler and Lederer (14) respectively Serum samples from some of the males in group C were kindly provided by O Mowé MD A/S Borregaard, Sarpsborg. Serum total cholesterol was determined on samples from groups E and F by the method of Carr and Drekter (5)

Cholesterol (Eastman Organic Chemicals) was dissolved, crystallized 4 times and used as cholesterol standard. Trioleine (Applied Science Laboratories) served as triglyceride standard, and the values are given relative to the molecular weight of 885.5 This gives results 3% above the equivalent molar concentration of triglycerides.

Phospholipids were determined as lipid phosphorus (8) and the values converted to phospholipids by multiplication by 25 Total serum lipids were calculated from the cholesterol, triglyceride and phospholipid values (6)

The methodological error expressed as the coefficient of variation between duplicate determinations on the same samples, was found to be 3% or 6–10 mg/100 ml

Table I *Serum lipid and lipoprotein-lipid values (mg/100 ml) in groups A–D*

Mean±S D range 95th percentile (in italics) and coefficient of variation (%) (within parentheses)

Group	Sex	Age (y)	*N*	Cholesterol	Triglycerides	Phospholipids	Total lipids	β-lipoproteins	Pre-β-lipoproteins	α-lipoproteins
A	♂	18–24	25	180± 4 145–230 *224* (13)	85±40 30–165 *164* (47)	10±29 168–280 *275* (14)	565±76 450–790 *775* (13)	305±66 205–450 *435* (22)	102±35 55–180 *178* (34)	158_79 85– 10 *704* (18)
B	♀	1–24	25	185±26 150–55 *245* (19)	70±28 25–135 *131* (40)	218±24 170–255 *252* (11)	570±66 445–665 *664* (1)	296±64 170–435 *425* (22)	103±28 90–155 *154* (77)	171±35 85–205 *202* (20)
C	♂	40–59	93	256±54 150–435 *355* (21)	133±57 58–462 *303* (43)	248±37 175–338 *323* (15)	794±151 516–1 233 *1 115* (19)	429±106 200–785 *595* (25)	164±95 40–575 *389* (58)	187±47 110–315 *273* (25)
D	♀	40–59	25	258±39 188–390 *340* (15)	100±37 55–172 *169* (37)	255±32 197–318 *316* (13)	743±113 535–1 007 *975* (15)	379±74 225–525 *519* (70)	111±45 60–225 *209* (41)	253±49* 180–390 *340* (19)

$p<0.01$ $p<0.001$ for difference in values between males and females of the same age

for both cholesterol methods 5% or 4–8 mg/100 ml for triglycerides and % or 4–7 mg/100 ml for phospholipids. The two methods for cholesterol determination were compared by analysing 80 different sera by both methods. The mean values from the two series were very similar (279 6 and 279 9 mg/100 ml). The triglyceride values obtained with the present method compare well with those obtained by Carlson and Lindstedt (4), as by the exchange of ten serum samples with dif levels of triglycerides.

Lipoprotein electrophor is Quantitative serum lipoprotein electrophoresis was performed in agarose gel as earlier described (13). The values for the lipid part of the lipoprotein complex (lipoprotein–lipid) of each class of the lipoprotein were estimated by multiplying the *value obtained for total lipids by the relative amount of* dye uptake in each fraction.

Low density lipoprotein (LDL) quantification. The approximations given (9 11) for LDL-cholesterol concentrations without the use of preparative ultracentrifugation were employed as follows: LDL-cholesterol=Total cholesterol—very low density lipoprotein (VLDL)-cholesterol—high density lipoprotein (HDL)-cholesterol where VLDL-cholesterol is taken as total triglycerides divided by 5 and HDL-cholesterol is arbitrarily given as 45 mg/100 ml.

Another measure of LDL-cholesterol was obtained by multiplying the electrophoretically determined β-lipoprotein by the cholesterol content, 41.5% (3) in this lipoprotein.

These two estimates were compared by regression analysis and the correlation coefficient was determined

Statistical analysis Comparisons between averages were performed by *Student t-test and the regression* line was obtained by the method of least squares

Table II *Serum total choleste ol concentrations (mg/100 ml) in groups E and F*

	Group E (males)			Group F (females)		
Age (y)	*N*	Mean±S.D	95th per centile	*N*	Mean±S.D	95th per centile
15–19	56	197±33	265	24	208±32	271
20–29	84	230±40	314	37	221±40	295
30–39	207	259±39	341	28	247±3	300
40–49	198	280±50	383	134	284±38	360
50–59	171	287±50	373	109	322* ±59	421
60–69	112	282±48	384	50	337 **±6_	448
Total	828			382		

** $p<0.001$ for the difference between males and females of the same age.

Table III *Comparison of serum total cholesterol concentrations (mg/100 ml) among four Scandinavian male population samples aged 40–59 years*

	No of subj.	Mean±S D.	% of population with cholesterol higher or equivalent 300	325	350	Mean+ 2 S.D	400
Present series							
Group C	93	256±54	18	9	5	4.3	—
Group E	369	284±50	26	20	10	4 0	1.6
Berge & Nicolaysen	6 357	270±51	26	13	6	[illegible]5	1 1
Carlson & Lindstedt							
Group 10	914	280±50	32	18	11	3 5	2.0

RESULTS

The results of the lipoprotein and lipid determinations for groups A–D are given in Table I and for groups E and F in Table II.

All lipid and lipoprotein concentrations showed an increase with age in both sexes Serum cholesterol reached its highest mean level in males (group E) in the age group 40–49 whereas it continued to increase in females (group F) up to a mean value of 337 mg/100 ml in the age group 60–69 Serum cholesterol concentrations above 400 mg/100 ml were found in 1.5 9 and 20% of the females (group F) in the age groups 40–49 50–59 and 60–69

No striking differences in cholesterol concentrations were found between males and females in the younger age groups in the small series (groups C and D) The difference between the two sexes with respect to the cholesterol value was however highly significant in the age groups higher than 50 years in groups E and F Highly significant differences ($p<0.001$) were also found in mean cholesterol concentrations between males in the selected and unselected series C and E and between the females in groups D and F The highest levels were found in the unselected series E and F

The percentage of males 40–59 years of age with cholesterol concentrations higher than certain values commonly used to define hypercholesterolaemia (300 325 350 and 400 mg/100 ml) are given in Table III and compared with results from two other Scandinavian investigations (1 4)

The distribution of some of the lipid and lipoprotein concentrations are illustrated in Figs 1–4 The distribution of triglyceride values in group C was distinctly skewed (Fig. 2) All these individuals were fasting and none had chylomicronaemia as judged from appearance of their sera and from electrophoretograms. Eight of the 12 subjects with triglyceride concentrations higher than 10 mg/100 ml were reinvestigated Their triglyceride levels were high also in the new samples

To evaluate the information obtained by lipoprotein quantification, the results in group C were further analysed None of the 93 subjects had elevated phospholipid or total lipid levels without increase in either cholesterol or triglyceride concentrations, using the 95th percentile as the upper limit By the same criterion (95th percentile) ele-

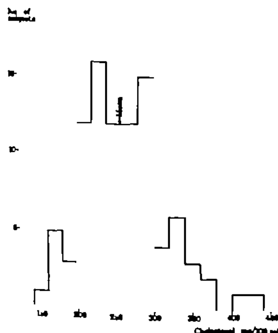


Fig 1 Distribution of serum cholesterol values in group C (93 males, 40–59 years).

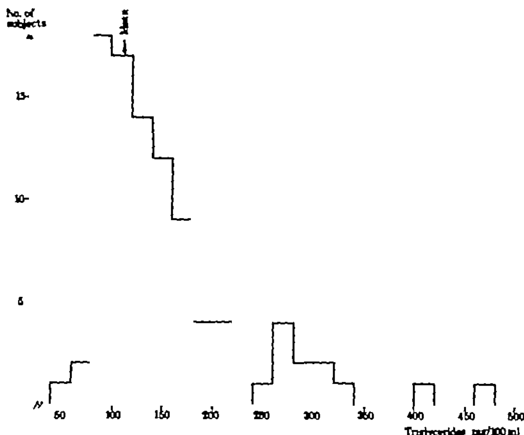


Fig 2 Distribution of serum triglyceride values in group C (93 males, 40–59 years)

... β- or pre β-lipoprotein concentrations were ... in 6 of 8 subjects with increased cholesterol or triglyceride levels, and a further 3 of the remaining 85 males had moderate rises in β- or pre-β-lipoprotein values without elevation of cholesterol or triglycerides.

The two estimates of LDL-cholesterol concentrations generally showed good agreement in the 168 subjects The correlation coefficient was found to be 0.84 and the regression equation was Y= 0 763X+30.2 where Y is the electrophoretically determined LDL-cholesterol and X the cholesterol value computed from the plasma triglyceride and cholesterol concentrations

Somewhat lower values were found for LDL cholesterol determined by the electrophoretic method The largest differences between the results from the two methods were seen with plasmas with increased amounts of triglycerides and pre-β-lipoproteins The 95th percentile for LDL-cholesterol by the "calculation method" based on serum triglycerides and cholesterol concentrations was found to be 179 156 279 and 267 mg/100 ml in groups A, B C and D

DISCUSSION

The serum lipid concentrations found in this study showed skew distributions most pronounced for triglycerides in males 40–59 years old (group C). Logarithmic transformation normalized the distribution of the lipid and lipoprotein levels with the exception of that of triglyceride concentration.

The lipid levels found in this study are in good agreement with those from other Scandinavian studies (1 4 7 15) The lipoprotein lipid levels, as obtained by densitometry of agarose gel electrophoretograms are similar to those reported by Dyerberg and Hjorne (7) and are also in good agreement with those found in ultracentrifugation studies after correction for the apoprotein content of the different apoproteins (10 12 16) This confirms the validity of the method employed

For all serum lipid and lipoprotein concentrations an increase with age was observed in groups

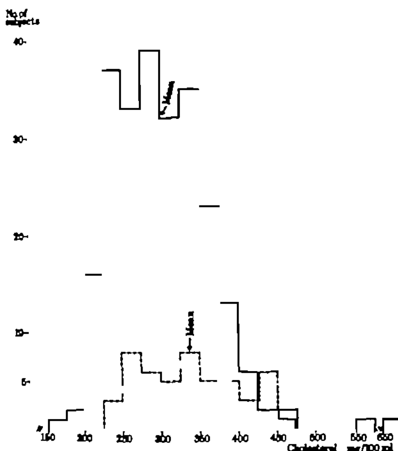


Fig. 3. Distribution of serum cholesterol values in 293 females (40–69 years) from group F. - - - = 50 females, 60–69 years —— = 243 females, 40–59 years.

A through D. For cholesterol the increase was also observed throughout in group F. The rise in cholesterol levels did not continue in males above 50 years of age (group E), a finding which also has been reported by other workers. Significant differences in lipid and lipoprotein concentrations between the youngest males and females (groups A and B) were not seen, but were evident between groups C and D where the triglyceride β- and pre-β-lipoprotein levels were higher in men and the α-lipoprotein level was higher in women.

The expected rise in β- and pre-β-lipoprotein levels with increasing cholesterol and triglyceride concentrations respectively was confirmed, but very few subjects had elevated β- or pre-β-lipoprotein fractions without concomitant rise in either cholesterol or triglyceride levels.

The wide range observed for cholesterol and other serum lipid concentrations could possibly be narrowed by quantification of separate lipoprotein fractions. However, the coefficients of variation for the separate lipoproteins were higher or similar to those observed for serum cholesterol and triglycerides.

The definition of a "biologically normal" upper limit for lipid and lipoprotein concentrations is not possible from the present data. The 95th percentile for each age and sex group may be utilized for an operational definition of "normality", despite the small numbers of subjects in several of the groups studied. However, the limitations of this pragmatical method must be kept in mind.

ACKNOWLEDGEMENTS

This work was supported by grants from the Norwegian Council on Cardiovascular Diseases, J. L. Tiedemanns Tobaksfabrik, Joh. H. Andresens medisinske fond and the University of Oslo, as well as by a gift from Det gjensidige forsikringsselskap Norske Liv.

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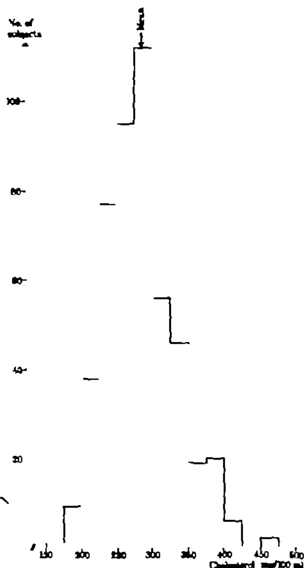


Fig 4 Distribution of serum cholesterol values in 481 males (40–69 years) from group E

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THE DIABETES SURVEY IN BERGEN NORWAY 1956

A Ten-year Follow up of an Epidemiologic Study and a Study of Blood Sugar Values Rel ted to Sex Age and Weight

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Abstract A follow-up study of 209 persons examined in the diabetes survey in Bergen in 1956 is discussed The material was divided into five groups on the basis of laboratory findings from 1956. *Latent diabetic* In 1956 diabetes as strongly suspected in most persons in this group. The follow-up study showed positiv diabetes in 1 of 3 persons. The average age of the men was 69 of the women 75 T used insulin and 3 peroral antidiabetics. In the remainder the disease could be kept under control by diet Diabetic complication of moderate degree ere observed in 7 persons. The mortality during the observation period was 60% for both sexes Cardiovascular disease was found in a large percentage of the deceased *Borderline cases* In this group diabetes as found to have dev loped n 8 of the 41 persons examined. The average age of the men was 65 of the women 70 The diabetics of this group had easily controllable diabetes The diagnosis is still uncertain in 46% of the group *Glycosuria non-diabetic* Diabetes as found in 5 of the 41 persons examined. *Screened posit non-diabetic* Diabetes was found in 2 of the 36 persons xamined. *Normal material* Diabetes was, somewhat surprisingly found 7 of the 68 persons examined. As the attendance at the follow up survey as only 70% it is possible that certain degree of selection had taken place. It can therefore hardly be assumed that the prevalence of diabetes in this group is representative of the development of diabetes in the constituency of Nygård during the observation period. The follow-up study lso showed considerable reduction in glucose tolerance during the observation period, the average levels of both fasting blood sugar and -hour blood sugar after admini tration of glucose showing clear increase in all diagnostic groups.

The first epidemiologic study of the prevalence of diabetes in a population group was made in 1946 by Wilkerson and Krall (13) Blood and urine were analysed and 40 know diabetics and 30 new cases were found in the group of 3516 persons ex amined i.e 0% diabetics in all This survey revealed that diabetes is more common than earlier assumed and that there is a large number of undiagnosed cases in the general population

Wilkerson and Krall also found a group of 118 individuals with abnormally high blood sugar le el who could not be classified as diabetics A follow up after 4 years (14) showed 17 new cases (14 4) in this high blood sugar group I a control group of 225 persons 4 diabetics were found (1 8%)

Since 1946 diabetes surveys have been carried out in a number of countries, particularly n America (1) but also in Europe (5) The prevalence of diabetes in these surveys has ried be tween 0.4% and 5.5% the number of new cases varying from 0 22% to 17%

In a follow-up study after years of 800 persons with high blood ugar levels Fisher and Mc Donald (3) found 8% new diabetics Only in very few cases (3 9 12) have studies been made to see how the disease develops in the newly discovered cases and of developments in persons w th high blood sugar levels or glycosuria who ould not be diagnosed as diabetics

In 1956 a diabetes survey was made in Nygård constituency Bergen Norway (6) in connection with a mass X-ray screening I 1966–68 follow up study was made of the persons who were found to ha e high blood sugar levels and/or glycosuria at the first examination, and of a normal material

RÉSUMÉ OF 1956 SURVEY

A total of 796 men and 4645 women above the age of 14 attended the 1956 mas X-ray screening, of whom

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Table I *Mortality during the observation period 1956–1966–68*

Diagnosis in 1956	Sex	N	Deaths n	Deaths %	Age at death (y.) Range	Age at death (y.) Mean
Latent diabetics	♂	28	17	60	47–81	68
	♀	42	25	60	68–89	81
Borderline cases	♂	41	14	34	47–84	73
	♀	41	15	37	62–87	75
Non-diabetics						
Glycosuria	♂	37	2	6	43–46	45
	♀	17	1	6	74	74
Screened positive	♂	26	3	11	57–76	67
	♀	28	7	25	58–86	72
Normal	♂	39	4	10	55–93	78
	♀	58	4	7	63–66	64
Total		357	92	23		

2 273 men and 3 657 women took part voluntarily in the diabetes survey

On the basis of predetermined screening levels for blood sugar or glycosuria, diabetes was suspected in 510 persons (259 men, 251 women). Of these 19 men and 21 women were earlier known diabetics under treatment. The remaining 240 men and 230 women were examined further with glucose tolerance tests 100 g glucose per os. A "normal material" of 194 persons was subjected to the same tests, to provide a basis of comparison for evaluation of glucose tolerance. The following criteria were used in grouping the material.

Diabetes was diagnosed if the fasting blood sugar was higher than 120 mg/100 ml or the 2-hour blood sugar higher than 180 mg/100 ml. The diagnosis of borderline case was made if the 2-hour blood sugar was between 150 and 180 mg/100 ml. If the 2-hour level was below 150 mg/100 ml the diagnosis of non-diabetic was made. Glycosuria was not considered necessary for a diagnosis of diabetes. The final diagnoses after glucose tolerance tests in 1956 were as follows: latent diabetics 28 men and 42 women, borderline cases 41 men and 41 women, non-diabetics 150 men and 122 women. There were 21 men and 25 women who did not attend the examination. Of the 510 screened positive 129 had glycosuria, of whom 54 were diagnosed as non-diabetics.

METHODS USED IN 1956 SURVEY

Urine sugar was tested with Clinitest tablets. Capillary blood sugar was analysed by Sørensen's modification of Dreywood's anthrone method (11). The height and weight of all attending were measured.

The anthrone method is suitable for mass surveys, but requires concentrated sulfuric acid and is thus extremely unpleasant. In 1966–68 the Hagedorn-Jensen reduction method for blood sugar determination was employed. A parallel study carried out in 1956 with the anthrone and Hagedorn-Jensen methods gave the result, after statistical analysis, that the fasting blood sugar levels obtained by the anthrone method were on an average 10.09 mg/100 ml higher and the 2-hour blood sugar levels in tolerance tests 11.71 mg/100 ml higher than by the Hagedorn-Jensen method. In recent years it has become more and more common to determine blood sugar by the glucose-oxidase method, which gives "true blood sugar". Eriksen et al (2) found in a parallel study that the Hagedorn-Jensen method gives levels 15 mg/100 ml higher than the glucose-oxidase method.

METHODS USED IN 1966–68 FOLLOW-UP

Plan of study

Urine was examined for sugar with Tes-Tape, for ketone bodies with Ketosticks, and for albumen with Albusticks.

Capillary blood sugar was determined by the Hagedorn-Jensen method. The following examinations were made: 1) Fasting blood sugar. 2) Morning urine was examined for sugar, ketone bodies and albumen. 3) Tolerance tests with 100 g glucose per os were carried out in all persons not being treated for diabetes. Double specimens of capillary blood were taken after 1 and 2 hours, the mean value of the analyses being entered as the result. 4) Clinical examination. All those attending the blood sugar survey were given a clinical examination by one of us (E. A. or S. R.) with a view to cardiovascular disease, neuropathies, retinopathies and nephropathies. All attendants were weighed.

Death certificates were obtained for all who had died during the observation period.

Criteria for diagnosis of diabetes

In a publication issued in 1965 (1) an expert committee appointed by the WHO gave criteria for the diagnosis of diabetes mellitus. The Committee stressed the desirability of standardizing examination methods and diagnosis criteria in order to reach a better basis of comparison for the results of various studies.

Table II. *Causes of death (main (M) and second (S) diagnosis) based on notified diagnosis to the Registrar of the Bergen Public Health Office*

Diagnosis in 1956	Total mortality	Diagnosis: Diabetes M	Diabetes S	Cardiovascular diseases M	Cardiovascular diseases S	Mors subita M	Other M	Other S
Latent diabetics	♂ 17		5	12	1	2	3	3
	♀ 25	5	4	11	11	1	8	3
Borderline cases	♂ 14		1	8	2	2	4	
	♀ 15		3	6	4		9	2
Non-diabetics	♂ 2			1		1		
Glycosuria	♀ 1						1	
Screened positive	♂ 3						3	1
	♀ 7			2	1		5	
Normal	♂ 4			3		1		
	♀ 4			1		1	2	

As to diagnosis, the Committee laid most emphasis on 2-hour blood sugar after peroral tolerance test, giving the following levels for capillary "true blood sugar" Levels below 120 mg/100 ml are normal. If levels are higher than 140 mg/100 ml, the diagnosis of diabetes can be made Levels between 120 and 139 mg/100 ml are defined as borderline levels. It is strongly recommended that this group (borderline cases) shall be re-examined at regular intervals. The Committee added: "When higher level is found in a person over 45 years of age, and especially over 65 the Committee considered that the other clinical data—family history physical examination, etc.—should provide the main guides to the diagnosis and treatment needed, since population-study data becoming available make it clear that aging is associated with diminishing glucose tolerance (i.e. higher blood-sugar curve)

Classification of material from the follow-up study in 1966–68 has been made in accordance with WHO diagnostic criteria, blood sugar levels found by the Hagedorn-Jensen method being converted to "true blood sugar"

Mortality during observation period 1956–1966–68

There was a considerable mortality within the material (Table I), among the latent diabetics 60% both for men and women.

Mortality among borderline cases was also high, 34% for men and 37% for women. Statistical analysis of these figures (carried out by L. Glazrodanner) showed that mortality rate was above normal in both latent diabetics and borderline cases, but that the difference was not statistically significant. In this connection it may be mentioned that Wilkerson and Krall (14) found considerably higher mortality among the known and newly diagnosed diabetics. Copies of death certificates were obtained for all deaths (Table II). Among the latent diabetics diabetes was given as the main cause of death for 5 women, and as contributory cause for 4 women and 5 men. Among the borderline cases diabetes was given as second diagnosis for 1 man and 3 women. From this it will appear that 30% of the dead females and 19% of the dead males in the groups latent diabetics and borderline cases were known to have diabetes at the time of death.

Cardiovascular disease dominated the list of causes of death. Among latent diabetics cardiovascular disease was given as the main cause of death in 23 of 42 and as a contributory cause in 12. In other words, in 35 of the 42 latent diabetics (83%) cardiovascular disease was known to be present at the time of death. If mors subita is included, the figure rises to 38 of 42 (90%).

In the group borderline cases cardiovascular disease was listed as the main cause of death in 14 of 29 cases, and as a contributory cause in 6. Twenty of the 29 borderline cases (69%) were thus known to have cardiovascular disease at the time of death. If mors subita is included the figure reaches 76%

MATERIAL OF FOLLOW UP STUDY IN 1966–68

It was our intention to examine all survivors among the 70 latent diabetics, 82 borderline cases and 54 persons with glycosuria from the 1956 survey

The group screened positive non-diabetics numbered

Table III. *Survivors at follow-up study in 1966–68*

Diagnosis in 1956	Males	Females	Total
Latent diabetics	11	17	28
Borderline cases	27	26	53
Non-diabetics			
Glycosuria	35	16	51
Screened positive	23	21	44
Normal	35	54	89
Total	131	134	265

Table IV. *Attendance at follow-up study in 1966–68*

Diagnosis in 1956	Attendants (n) Males	Females	Age of males (y.) Range	Mean	Age of females (y.) Range	Mean
Latent diabetics	9	14	61–74	69	55–85	75
Borderline cases	1	20	4_–86	65	5_–80	70
Non-diabetics						
Glycosuria	30	11	25–71	57	3_–71	57
Screened positive	18	18	38–77	55	28–87	56
Normal	29	39	25–86	50	40–89	57
Total	107	102				
Both sexes	209					

277 persons in the original material. Of these each fifth person w_ picked out by random sampling so that 54 of this group took part in the follow-up study. Half of the "normal material" group from 1956 was selected in a similar manner giving a group of 97 for the follow-up study.

Table III shows the distribution of the survivors in 1966–68 in the various diagnostic groups, and Table IV the attendance at the follow-up study by sex and age. Of the 26_ summoned 209 presented for examination.

RESULTS OF FOLLOW UP STUDY IN THE DIFFERENT DIAGNOSTIC GROUPS

Table V shows the fasting blood sugar levels and Table VI the 2 hour blood sugar levels for men and women in the different diagnostic groups in 1956 and 1966–68. The levels found by the anthrone method in 1956 have been converted to Hagedorn Jensen levels.

Table V. *Fasting capillary blood sugar levels in 1956 and in 1966–68 (Hagedorn Jensen method)*

		Capillary blood sugar (mg/100 ml) <120	120–129	130–139	140–149	150–159	160–180	>180	Total
Males									
Latent diabetics	1956		4		2		1		9
	1966–68	5			1	1	1	1	9
Borderline cases	1956	19	1	1					1
	1966–68	16		1					17
Non-diabetics									
Glycosuria	1956	29	1						30
	1966–68	27	1			1	1		30
Screened positive	1956	18							18
	1966–68	18							18
Normal	1956	28	1						29
	1966–68	25	1	1					29
Female									
Latent diabetics	1956	7	2	1	2	1	1		14
	1966–68	7	1	2		1	1	2	14
Borderline cases	1956	20							20
	1966–68	17	1	1	1				20
Non-diabetics									
Glycosuria	1956	11							11
	1966–68	11							11
Screened positive	1956	18							18
	1966–68	17		1					18
Normal	1956	39							39
	1966–68	36		1					39

Determination failed in persons.

Table VI. Glucose tolerance test with 100 g glucose per how apillary bl od ugu les ls n 1956 and in 1966–68 (H ged rn-Jensen method)

		Capillary blood sugar (mg/100 ml)							
		<120	120–129	130–139	140–149	150–159	160–180	>180	Total
Males									
Latent diabetics	1956					1	2	6	9
	1966–68		1		1			5	7
Borderline cases	1956		1	3	8	5	3	1	1
	1966–68	3				1	5	5	20
Non-diabetics									
Glycosuria	1956	19	9						30
	1966–68	13	9				3	1	30
Screened positiv	1956	14	4						18
	1966–68	11		3	1	1	1	1	18
Normal	1956	17	7	1		1		1	29
	1966–68	14	4		1	1	5	3	25
Females									
Latent diabetics	1956						6		13
	1966–68					1	1	10	1[illegible]
Borderline cases	1956		2		6	4	5	1	20
	1966–68	3	1		1	4	4	4	19
Non-diabetics									
Glycosuria	1956	7	4						11
	1966–68	3		4	1		1		11
Screened positiv	1956	11	3	4					18
	1966–68	6			3	4	1		15
Normal	1956	26	5	3					34
	1966–68	9	4	6	7	3	5	3	37

Determination failed in persons.
Determination failed I 1 person.

Latent diabetics

Se en men of 9 examined nd all 14 examined omen were now diagnosed as positive diabetics In male persons the diagnosi of diabetes could not be confirmed

The age within the group was high (Table IV) In most cases the disease could be classified as a relati ely mild old-age diabetes Two used insulin and 3 peroral antidiabetics In the remainder the disease had been kept under control by diet Clinical examination showed moderate diabetic complications in 4 men and 3 women (angiopathy polyneuritis 1 retinopathy 1 nephropathy cataract and arteriosclerosis obliterans 1)

Borderline c se

This is the group described by WHO s Committee s 'border-line state in which a relatively high development of diabetes can be expected The final diagnosis at the f llow-up examination showed that 4 men of 1 examined and 4 women of 20 examined were positi e diabetics N ne men and 10 women were still classified as borderline cases and 8 men and 6 women as non-diabetic Moderate neuropathy was found 1 wome The follow-up study confirms the expectation of high rate of development in this group and that per sons with blood sugar level in the borderline area between definitely normal and pathologically high should be kept under supervision.

Glyco uria

Four men of 30 examined and 1 woman of 11 ex amined had developed diabetes One male person is classified as borderline case Twenty-five men and 10 women have been diagnosed as non-diabetics No diabetic complications ha been demonstrated The study ndicated that findings of glycosuria should not be brushed aside e e if

Table VII *Height/weight ratio in 1956 and in 1966–68 (Natvig's tables)*

		Underweight (%)				Overweight (%)				
		>20	20–16	15–10	<10	<10	10–15	16–20	21–25	>25
Males										
Latent diabetics	1956						3	2		2
	1966–68				3	2	3			1
Borderline cases	1956				4	7	3	1		4
	1966–68		2	2	3	9		2		3
Non-diabetics										
Glycosuria	1956		1	4	11	10	4			
	1966–68	3	1	4	8	12	2			
Screened positive	1956	1		1	5	9	1	1		
	1966–68	1		2	4	8	2			1
Normal	1956		1		10	12	1	2	2	1
	1966–68	1		1	10	12	1	2	2	
Females										
Latent diabetics	1956				1	4	3		1	5
	1966–68				3	4	3			4
Borderline cases	1956			1	6	3	1	3	2	4
	1966–68		2	1	4	7		2	2	2
Non-diabetics										
Glycosuria	1956				4	3	1			
	1966–68				4	1	1	1	2	2
Screened positive	1956	1		2	6	6	1	1	1	
	1966–68		1	3	5	3	1	2	2	
Normal	1956			2	12	16	2	2		1
	1966–68	2	2	1	13	12		1	2	4

Determinations missing in 3 persons.
Determination missing in 1 person.

blood sugar levels are normal. Persons with glycosuria should be examined at regular intervals.

Screened positive

This group showed abnormally high blood sugar levels at the first screening, but glucose tolerance tests gave normal levels in 1956.

The result of the follow-up survey showed that of 18 men and 18 women examined 2 men had developed diabetes. The others were non-diabetics. No diabetic complications were demonstrated.

Normal material

In 1956 the normal material was selected by random sampling among those who had shown a negative reaction to the chosen screening levels. In 1966–68, as earlier mentioned, half of the group was selected for follow-up examination.

Of 29 men examined 4 were diagnosed as diabetics, 4 were classified as borderline cases and 21 as non-diabetics. Of 39 women examined 3 were diagnosed as diabetics, 6 were classified as borderline cases and 30 as non-diabetics. This result is astonishing. The age of the group, however, is high (Table IV). Attendance at the follow-up examination was only 70%. The possibility that this was to some degree a selected portion of the group cannot therefore be disregarded.

For this reason it can hardly be assumed that the prevalence of diabetes in this group is representative of the development of diabetes in the constituency of Nygård during the observation period, especially if a comparison is made with the group screened positive non-diabetics in which the prevalence would be expected to be higher than in the normal material.

Height/weight ratio during observation period

The height/weight ratio in 1956 and in 1966–68 for men and women appears from Table VII. No major changes took place in the height/weight

Table VIII. *Fasting mean capillary blood s ga level (mg/100 ml) (Hagedorn-J nsen m thod) ith S.D in 1956 (x) and in 1966–68 (y)*

			Non-diabetics		
	Latent diabetics	Borderline cases	Glycosuria	Screened positive	Normal
Males (N)	9	19	30	18	29
Mean (x)	129 0± 7 0	103 4±2.3	91 1±1 9	93 9±1.2	91.2±1.8
S D	21 1	9 9	10.2	5 1	9 8
Mean (y)	135 1±14 9	109 0±2.7	101.8±3.6	102.3±2.4	101 7±2.6
S D	44.8	11.6	19 6	10 2	13 9
Percentile for rxy	r<p 90	>p 99	>p 99	>p.90	r=p 99
Females (N)	14	20	11	18	39
Mean (x)	121.5± 7.3	98 6±1.9	91.3±1.9	90.4±1.5	88 1±1
S D	27 4	8.3	6.4	6.4	7.3
Mean (y)	132.8±11.8	106.1±3 4	96 6±1 7	103.5±2.7	98 6±1 7
S D	44.3	15 4	5.8	11.3	10.6
Percentile for rxy	r<p 90	<p 90	>p 95	r<p 90	r<p 90

ratio during the observation period. A slight displacement towards a higher weight can be seen in females in the groups glycosuria, screened positive and normal

Changes in glucose toleranc during observation period

A number of studies have shown that glucose tolerance decreases with age (4 6, 7 10) This was also clearly apparent when the diabetes material from 1956 was analysed as there was a definite statistical increase with age in the average levels of both postprandial blood sugar and blood sugar after glucose tolerance tests This reduction in glucose tolerance was present both in normal weight and overweight groups and can thus not be ascribed to the general weight increase with age

The changes in glucose tolerance for the material in the years 1956–1966–68 appear in Tables

Table IX. *Glucose tolerance test ith 100 g glucose per os 2-hou mean capillary blood sugar level (mg/100 ml) (Hagedorn-Jensen method) with S.D in 1956 (x) and in 1966–68 (y)*

			Non-diabetics		
	Latent diabetics	Borderline cases	Glycosuria	Screened positive	Normal
Males (N)	7	20	30	18	28
Mean (x)	184 0± 9 7	150 9±3 4	108 3±4 1	108.6±4.2	114.7±4 1
S.D	25 7	15 4	22 2	17.8	21.5
Mean (y)	228.7±24.5	153 1±8.9	1 4 0±6.3	118.8±7.3	126 3±7.8
S.D	64 8	39 9	34 4	31 1	41.5
Percentile for rxy	<p 90	r<p 90	<p 90	r<p 90	>p 99
Females (N)	13	19	11	18	37
Mean (x)	200.4±12.1	148.6±3.6	111 4±3.3	116 2±3 1	115.7±2.9
S.D.	43 7	15 7	13.3	13.3	17 7
Mean (y)	238 4±20.4	158.5±9.3	129 0±5 3	134 7±4.9	138.7±4 7
S.D.	73 7	40.7	17 7	20 9	28.3
Percentile for rxy	r<p 90	r<p 90	<p 90	p 90<r<p 95	>p 99

VIII and IX Table VIII shows the average levels for fasting blood sugar and Table IX the average levels for 2-hour blood sugar with S D for men and women in the various diagnostic groups for the years 1956–1966–68 The anthrone method levels from 1956 have been converted to Hagedorn-Jensen levels Both mean value of fasting blood sugar and 2-hour blood sugar show an increase in all diagnostic groups both for men and women. The difference is statistically significant for fasting blood sugar for male borderline cases men and women with glycosuria and men in the normal material For 2 hour blood sugar the difference is statistically significant for the men and women in the normal material

The study shows that the fasting blood sugar value increased in all groups in the course of a ten-year period by an average of 9 mg/100 ml (5–13 mg/100 ml) and the 2-hour blood sugar value by an average of 18 mg/100 ml (3–44 mg/100 ml) The difference was greatest in the group latent diabetics in which the increase was 44 and 38 mg/100 ml for men and women respectively If this group is disregarded the increase in the remaining groups is 13 mg/100 ml (3–23 mg/100 ml) At the same time we see that the S D values have increased in the course of the observation period The 1956 survey showed that glucose tolerance decreases with age The follow-up study shows for the age groups the material represents increase during a ten-year period will be oximately 9 and 13 mg/100 ml for fasting d 2-hour blood sugar values respectively

DISCUSSION

Hayner et al (4) have demonstrated that with increasing age blood sugar levels 1 hour after oral glucose loads rise steadily by approximately 13 mg/100 ml each decade from 113 mg/100 ml in the thirties to 173 mg/100 ml in the eighties We know neither the reason for the reduction in glucose tolerance registered with increasing age nor the effect it may have on the development of other diseases Many theories have been proposed Hayner et al consider that the reduction affects everyone and is thus simply an effect of old age

The large dispersion of levels for both fasting and 2 hour blood sugar in our material and the fact that dispersion increased during the observation period, argue against the theory that the reduction in glucose tolerance affects everyone Eriksen et al (?) have carried out a health survey which included a glucose tolerance test of 410 70-year-olds According to WHO s criteria 7.8% of this material were diabetics 19% latent diabetics and 17.3% borderline cases 35._% had normal glucose tolerance tests Eriksen et al found that the frequency of abnormal glucose tolerance tests increased with increasing occurrence of other abnormal examination findings Among healthy 70-year-olds 79% had normal glucose tolerance This study does not appear to indicate that there is a general reduction of glucose tolerance with age and Eriksen et al assert that WHO s criteria for the diagnosis of diabetes should also apply to the elderly Kaufman et al (7) also concluded from their material that the reduction of glucose tolerance is not evenly distributed in the population, and can thus not be ascribed to age They suggested that the reduction might be due to the occurrence of an inherited disposition to diabetes in the population This is a question which should be resolved as it would be of decisive importance for our criteria for the diagnosis of "old-age diabetes and thus also for treatment Keen et al (8) have shown that there is a clear relationship between reduced carbohydrate tolerance and frequency of cardiovascular complications

Is there a connection here or are we dealing simply with two parallel processes both due to increasing age? Only long-term studies of a relatively large population group can solve this problem

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ESTIMATION OF SERUM THYROTROPIN (TSH) AND STIMULATION WITH THYROTROPIN RELEASING HORMONE (TRH) IN THYROID DISEASES

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Abstract Serum TSH has been estimated using radioimmunological method in 128 controls: 1 9±1 7 µU/ml (mean ±S.D) in 97 hyperthyroid patients: 1.5±1.5 µU/ml, and in 40 patients with primary hypothyroidism: 116±72 µU/ml. After stimulation with 200 µg TRH [illegible] positiv response (Δ TSH>2.0 µU/ml) was found in 58 of 60 controls but only in one of 56 hyperthyroid patients. In 23 hypothyroid patients examined with the TRH test an exaggerated TSH response was found. Four clinical euthyroid patients with Hashimoto thyroiditis had moderately elevated serum TSH and an increased response to TRH whereas 9 euthyroid patients with diffuse goitre did not differ from the control group. During antithyroid treatment of 66 patients serum TSH was elevated only in half of the patients with subnormal serum thyroxine and normal in almost all cases with normal serum thyroxine. In 34 patients with primary hypothyroidism treated with l thyroxine serum TSH was elevated in all with subnormal serum thyroxine and was not normalized until serum thyroxine levels were within the upper part of normal range. Estimation of serum TSH is of major diagnostic value in primary hypothyroidism and valuable aid in the control of treatment of hypo- and hyperthyroidism. The TRH stimulation test is of value in the diagnosis of hyperthyroidism.

Since 1965 it has been possible to measure serum thyrotropin (TSH) by radioimmunological methods (26 36) Using these methods different investigators have found considerably elevated values of TSH in patients with primary hypothyroidism. Although the TSH concentration in patients suffering from hyperthyroidism has generally been found to be lower than in healthy persons in most laboratories it has not been possible on the basis of a single TSH estimation to distinguish between hyperthyroidism and euthyroidism (12 14 26 27 30 36)

The TSH secretion of the pituitary is stimulated by thyrotropin-releasing hormone (TRH) which is secreted from the hypothalamus In 1969 TRH was identified (3 31) as a tripeptide pyroglutamyl histidyl-prolinamide and shortly thereafte[illegible] synthesis became practicable (8 9) The effe[illegible] of TRH on the release of TSH is inhibited by thyroxine and triiodothyronine (5 33) and the e[illegible]fect of TRH on the TSH response therefore [illegible] dependent on a normal function both of the thyroid and of the pituitary gland. Thus, several studies have shown a lack of TSH response after TRH stimulation in some patients suffering from pituitary disorders (7 13 18) as well as in patients with hyperthyroidism (14 18 28). We have studied the diagnostic value of serum TSH estimations before and after stimulation with TRH in controls and in patients with thyroid diseases Furthermore serum TSH has been followed during antithyroid treatment and treatment of hypothyroidism.

MATERIAL

The control group consisted of 128 persons without thyroid diseases, 67 men and 61 women aged 18–87 [illegible]verage 49.3 years. The TRH stimulation test was performed in 60 of them (28 men and 32 women aged 16–87 [illegible]erage 46.5 years). The group with untreated hyperthyroidism consisted of 97 patients, 85 women and 1[illegible] men aged 14–88 [illegible]verage 51.8 years. The TRH test was performed in 56 of these patients (51 women and 5 men aged 14–84 [illegible]verage 54.8 years). In 66 patients with hyperthyroidism serum TSH was measured during long-term antithyroid treatment. For 41 patient this was the first attack, for 25 the second or more attack of hyperthyroidism. The patients had been treated from [illegible] weeks to [illegible] years, mean 8 months, and none [illegible] thyroid hormone Serum TSH was determined [illegible]

Table 1 *Serum TSH (μU/ml) in cont ols and patients with hyperthyroidism hypothyroidism and diffuse non-toxic goitre*

		x̄	S D	Range	95% limits
Euthyroid	128	1 9	1 7	0– 11 4	0– 4 8
Hyperthyroid	97	1 5	1.5	0– 8 7	0– 3.5
Hypothyroid	40	116	72	26–350	36–290
Diff se non-toxic goitre	14	1 3	1.5	0– 5 0	–

tients with primary hypothyroidism (35 women and 5 men aged 13–83 verage 59 6 years) and 23 of these patients (21 women and men aged 13–83 average 59.5 years) were tim lated with TRH In 34 patients suf fering from primary hypothyroidism serum TSH was studied during treatment. Serum TSH was also measured in 14 patients with diffuse non-toxic goitres and in 9 of them the TRH test wa performed. Finally 4 women with Hashimoto thyroiditis were examined

METHODS

The thyroid function was evaluated on the basis of serum thyroxine according to the method of Murphy T_3-resin uptake (Triosorb Abbott) and the 4 and 24 h ^{131}I uptake in the thyroid gland possibly with TSH stimulation or triiodothyronine suppression of the ^{131}I uptake Serum TSH was determined by radioimmunoassay with a modification of Wide s solid phase hnique (39) Human TSH from National Pituitary cy National Institute of Health Bethesda, USA labelled with ^{125}I (The Radiochemical Centre sham) according t H nter and Greenwood (15) labelled TSH was purified during a passage through G 100 Sephadex column (K 9/60 Pharmacia) The antibody against human TSH was obtained from National Institute of Health After the immunoglobulins had been precipitated with 18% Na_2SO they were fixed to cyanobromide-activated microcrystalline cellulose (Merck) Human TSH 68/38 from National Institute for Medical Research London, was employed for the standard TSH was dissolved in 0 05 M phosphate and 0.15 M NaCl buffer with 1% bovine albumin (Behring Werke) with pH 7 5 T 0 1 ml of the standards 0.1 ml O-serum was added The 'O-serum was collected from euthyroid persons who had been suppressed with 120 μg T daily for 7 days. T 0 1 ml of serum samples 0 1 ml of the buffer was added About 40 000 cpm of ^{125}I-labelled TSH was added both to the serum samples and t the standards. Finally 1 0 ml of the antibody suspension in the above mentioned buffer with 0.5% Tween 20 (Koch-Light Labora tories) and 1 U human choriongonadotropi (HCG) (Wellcome) was added HCG was added in order to saturate antibodies to TSH LH FSH and HCG After rotation of the mixture for 3 days at room temperature the tubes were centrifuged and the supernatant removed by suction The centrifugate w s washed 3 times with 0 ml 0 30 M N Cl with 0.5% Tween 20 and finally counted in a well scint llation counter All analyses were done in duplicate. A standard curv and three known serum samples were run every day

The lowest serum TSH alue which w significantly different from 0 was 1 0 μU/ml and the recovery of added human TSH to 10 different sera w s 98%+10% The coefficient of variation as found by repeated determinations of the same sample 20 times on consec utive days was within the normal range 15% and for the sample with high TSH content 12%

The TRH stimulation test was performed i the morning. The patient were not fasting A permanent drop with 0 9% saline was applied and at zero time 200 μg TRH was injected intravenously Blood samples for TSH estimation were drawn at 0 20 60 and 120 min. TRH was supplied by F Hoffman-La Roche & Co and Novo Industri A/S No difference bet een the preparations was found.

Statistics Student *t*-test was used except for materials including less than 25 persons. In these cases Wilcoxon s test was employed

RESULTS

The TSH values were below 4 9 μU/ml in 95% of the 128 controls (Table I) In 36% serum TSH could not be detected (<1 0 μU/ml) No dif ferences between the sexes could be demonstrated (Table II) The relation to age was investigated by dividing the controls into three age groups below 40 years (average 26 4) 40–59 years (average 49.5) and above 60 years (average 68.8) (Table II) Serum TSH levels were found to increase with age though a significant difference was demonstrated only between men below 40 and above 60 years ($p<0$ 05)

The mean maximal response of serum TSH to TRH is given in Table III In all but tw of the controls a rise in serum TSH (i e maximal re sponse >2 0 μU/ml) could be demonstrated The maximal value of serum TSH was reached in 43 persons 20 min following the TRH injection, in 1 persons after 60 min and in 5 persons the maximum was not reached until after 120 min However the actual difference between the TSH values at 60 and 120 min in these persons was very small No difference in the response to TRH between the sexes was found (Table II) Age dependent changes in the TSH response to TRH were evaluated by dividing the persons studied into three groups of mean age 24 3 50.3 and 71 5 years respectively (Table II) A significant

Table II *Serum TSH (µU/ml) and mean maximal TSH response (Δ Max TSH) to 200 µg i TRH in control*

Age (y)	Males				Females			
	Baseline TSH		ΔMax. TSH		Baseline TSH		ΔMax. TSH	
		Mean±S D.		Mean±S D		Mean±S D		Mean±S D
<40	.3	1.2±1.3	14	6 3± 9	14	1 6±1.5	11	7 4±3
40-59	4	1 9±..3	7	8.3±3 9	4	1 9±1 3	8	7.5±5 0
>60	20	2.3±1.6	7	..9±1 6	.3	5+1 9	13	8 9±7 5
Total	67	1.8±1.8	28	5 9±3 5	61	0±1 6	3..	8.0±5.6

age-dependent decrease in the serum TSH response to TRH was demonstrated only in men ($p<0.05$). Only a slight correlation between the basal TSH value and the maximal response to TRH was found ($r=0.27$ $p<0.05$) The reproducibility of the TRH test was estimated by repeating the TRH stimulation with an interval of at least one week in 7 persons The mean rise in serum TSH 20 min afte TRH injection was 4.5 µU/ml with S D of 1 6 µU/ml calculated from the duplicate estimations The corresponding values at 60 and 120 min were 3 0±1 3 and 1 1±1.. µU/ml. Judged by the Wilcoxon test for paired data no significant differences between the TSH responses were found ($p>0.10$)

In 97 patients w th untreated hyperthyroidism mean serum TSH wa found to be significantly lower ($p<0.05$) than in the control group The results are seen in Table I In 47% of the patients serum TSH was below 1 0 µU/ml The TRH test was performed in 56 of the hyperthyroid patients (Table III) Only one of these responded with an increase in serum TSH of more than 2 0 µU/ml after 120 min

In all the patients w th primary hypothyroidism serum TSH was found to be considerably ele ated and an exaggerated response to TRH was found in all patients in whom this test was performed

The patients suffering from Hashimoto thyroiditis were clinically euthyroid and had serum thyroxine w thin the normal range (mean alue 6 7 µg/ml) Serum TSH was moderately elevated in all of them and after TRH stimulation a considerable increase in TSH was observed Serum TSH was found to be normal n the patients with diffuse non-toxic goitre and the TRH test showed normal response of serum TSH in these patient

The results from the patients during long term antithyroid treatment are shown in Fig. 1 In cases w th subnormal serum thyroxine levels serum TSH was elevated only in 57% In patient w th normal serum thyroxine values serum TSH was found to be normal except in cases. Serum TSH estimations from 34 patients with primary hypothyroidism receiving l-thyroxine treatment are shown in Fig. .. Serum TSH w found to be elevated in all cases with subnormal serum thyroxine values among cases with normal serum thyroxine below 9 µg/100 ml serum TSH was elevated n 73% but only in 16% with serum thyroxine above 9 µg/100 ml

Table III *Serum TSH (µU/ml) in esponse to 200 µg i v TRH i ontrols and patients with hyperthyroidism hypothyroidism Hashimoto thyroiditis and diffuse non-to ic goitr*

		Baseline TSH (Mean±S.D)	ΔMax TSH Mean±S D	Range	p*
Euthyroid	60	2 0± 1 8	7 1± 4 8	0 6– 23 7	<0.001
Hyperthyroid	56	1 1± 1 3	0 3± 1 0	0 7	0 1>p>0 05
Hypothyroid	23	125 ±90	144 ±149	9 541	<0.001
Hashimoto	4	14 9± 8..	54 ± 57	6 1 1	
Diffuse non-to ic goitre	9	1 0± 1.5	7 4± 5 1	9– 19 7	0.01>p>0 001

Significance of the difference between serum TSH before and the maximal TSH alue after TRH tim

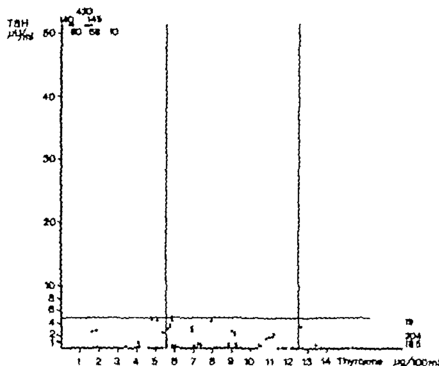


Fig 1 Relation between serum TSH and serum thyroxine in patients with hyperthyroidism during antithyroid treatment (131 samples from 66 patients).

DISCUSSION

Our results regarding normal values of serum TSH are in general agreement with several other studies though our data probably cannot be directly compared to those of other laboratories due to differences in the radioimmunoassay used (12 14 19 26 27 30 36 38) Like Lemarchand ud et al (23) and Odell et al (25) no dif in serum TSH between the sexes could monstrated Serum TSH was found to increase with age though significantly only in males These findings are in accordance with previous studies by Lemarchand-Béraud and Vannotti (24) whereas other investigators have found no age-dependent changes in serum TSH levels (34)

In studying the response of serum TSH to TRH different doses of TRH have been used (10 17 20 29 34 37) Most authors agree that little diagnostic information is gained by increasing the dose of TRH above 200 µg i v and in the present study this dose has been used as a standard procedure The maximum rise in serum TSH after stimulation with TRH in most cases occurred 20 min following TRH This finding is in agreement with most authors (1 11 14 18) We found that following serum TSH for more than 60 min after TRH injection no further information was obtained in these disorders In 2 of the controls a significant increase in serum TSH after TRH stimulation could not be demonstrated We have no explanation for this lack of response but similar results are described by others using a standard TRH stimulation (1 5) We have been unable to confirm the results of Jensen and Weeke (16) who found a high positive correlation between baseline TSH and the response to TRH The reproducibility of the TRH test in the recent study was in reasonable agreement with other studies (10 34) We could not demonstrate any sex dependent difference in the response to TRH which accords with Snyder and Utiger (34) but is in contrast to Ormston et al (28) In spite of increasing serum TSH the response to TRH diminished significantly with increasing age in males, while no age-dependent alterations could be demonstrated in females Our finding of a sex difference in age-dependent TSH response to TRH is in accordance with Snyder and Utiger (34 35)

In our studies of patients with thyroid diseases we found a significant difference in serum TSH between the control group and the group of patients with untreated hyperthyroidism but the overlap in serum TSH values between the groups was so great that a single determination of serum TSH is of no value in the diagnosis of hyper

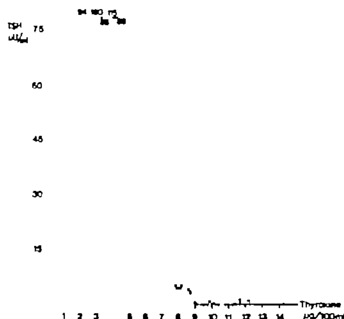


Fig. 2. Relation between serum TSH and serum thyroxine in patients with primary hypothyroidism during treatment with l-thyroxine (79 samples from 34 patients).

thyroidism. The TRH stimulation test, however allowed a good separation between euthyroids and patients with hyperthyroidism, as only one of 56 hyperthyroid patients had a response greater than 2.0 μU/ml and only two of 60 controls responded with less than 2.0 μU/ml. Our results concerning the diagnostic value of the TRH test in hyperthyroidism are in accordance with several authors (10, 14, 18, 28). Like in the patients of other investigators (6, 10, 14, 18, 28) a considerable elevation both in serum TSH and in the response to TRH was found in all the patients suffering from primary hypothyroidism. In these patients the TRH test offered no further diagnostic information than a single TSH determination. In the 4 patients with Hashimoto thyroiditis serum TSH was moderately elevated and they showed an exaggerated response to TRH although they were clinically euthyroid with serum thyroxine within the normal range. They were probably in a state of preclinical hypothyroidism (21, 28). The patients with non-toxic diffuse goitre did not differ from the controls either in serum TSH or in the response to TRH. These findings are in agreement with those of Beckers et al. (2).

During antithyroid treatment (Fig. 1) serum TSH was found to be elevated only in half of the patients with subnormal serum thyroxine values. This is probably explained by the finding of normal serum triiodothyronine levels in these patients (3). In the patients suffering from primary hypothyroidism treated with l-thyroxine (Fig.) serum TSH was elevated in 11 cases in which serum thyroxine values were below the normal range. With increasing serum thyroxine values we found in agreement with Cotton et al. (4) that serum TSH generally is not normalized until serum thyroxine levels are within the upper part of the normal range. In spite of serum thyroxine levels of the same order it thus appears that serum TSH is higher in patients with treated hypothyroidism than in patients during antithyroid treatment. The lower level of serum TSH in patients during antithyroid treatment might be explained by supposing that these patients have relatively higher serum level of triiodothyronine than those with hypothyroidism treated with l-thyroxine (22).

It can be concluded that a single serum TSH estimation seems to be sufficient in the diagnosis of primary hyperthyroidism. To avoid undertreatment of primary hypothyroidism, estimation of serum TSH also seems to be of value. The finding of elevated values of serum TSH in patients during antithyroid treatment must be considered as sign of overtreatment. In hyperthyroidism a single TSH estimation is of no diagnostic value whereas the TRH stimulation test must be considered a useful diagnostic supplement to other thyroid function tests.

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SERUM CALCITONIN IN MEDULLARY THYROID CARCINOMA

Radioimmunoassay Technique and Diagn tic Value

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Abstract A radioimmunoassay for the determination of human calcitonin in 200 µl aliquots of serum has been developed using rabbit antiserum against extract of an amyloid-producing medullary carcinoma of the thyroid gland (MCT). The assay was specific for human synthetic and nati calcitonin and sensitive enough to determine normal and subnormal serum levels. No cross reaction occurred with human or bovine parathyroid hormone. The levels (x̄±2 S.D) were 0 70±0.34 ng/ml in 115 control subjects, confirming that calcitonin is a normal serum peptide High calcitonin levels, >800 ng/ml, were found before surgery in 1 patients with verified MCT and low levels, ≤0 40 ng/ml, after thyroidectomy in 7 of 9 patients thyroidectomized for thyroid tumours other than MCT Our results show that this calcitonin assay has linical value in the diagnosis of MCT both primary and metastasizing. It is therefore of value also in the postoperative control of these patients. The levels of calcitonin correlated ell with the clinical estimation of the extent of the disease

The first radioimmunoassay of human calcitonin (h-CT) was published in 1969 (3) and allowed measurement only of elevated serum levels In 1970 Tashjian et al (10) reported an immunoassay which permitted the measurement of normal levels They applied their assay to three families with medullary carcinoma of the thyroid gland (MCT) (7 10)

We have developed sensitive radioimmunoassay of h-CT which measures normal and low levels in untreated serum The aim of this paper is to present the properties of our assay of h-CT and its clinical usefulness in subjects with MCT known since 1968 to produce calcitonin (10)

MATERIAL AND METHODS

The patient material consisted of 1 patients with amyloid-producing MCT verified by histopathology Most of them were found by screening for MCT in families w th Sipple yndrome bilateral MCT combined ith phaeochromocytomas syndrome inherited as an autosomal dominant trait (9) The other patient were diagnosed by fine needle aspiration biopsy of thyroid bnormalities, routine diagnostic method in our departments (8). A few patients ere operated pon only because of raised S-h-CT in the others her were additional indications for surgery such L nodules in the scintiscan of patient ith heredit Sipple yndrome and/or positi MCT cytology

The control consisted of 94 health adult healthy children, aged 1–19 years, and 9 patients h had previously been subjected to surgically tot l thyroidectomy for thyroid carcinomas other than MCT

Iodination and immunoassay standard of h-CT

Synthetic h-CT M from Ciba was used for labelling with N 125-I (Amersham England). A modification of the chloramine T method of Greenwood and H nter (4) was sed. The synthetic calcitonin was also used as reference standard The quality of the labelled antigen was tested by chromatoelectrophoresis (2)

Calcitonin-free erum

This was obtained from female 90-year-old patient, who had been completely parathyroid- and thyroidectomized for highly differentiated mixed papillary and follicular thyroid carcinoma and primary hyperparathyroidism. She should have no calcitonin-producing cells left and ga es pool of serum This serum was always dded to all tubes of the standard cur in aliquots of 200 µl of 1 10 dilution of her serum, corresponding to the average amount of serum proteins in unknow serum samples

Production of antibodie to human calcitonin

Antibodies were prepared by injection of six rabbits and five guinea pigs with extracts of human MCT The glands were freeze-dried, defatted, extracted in 6 M urea 0 01 M HCl-0.05 M cystein following the procedure of Aurbach (1) up to the second precipitation step Initial immunization was obtained by direct injection into spleen and lymph nodes of the extract absorbed on Carbon Statex E L (Columbian International Ltd.) together with complete Freund' adju-

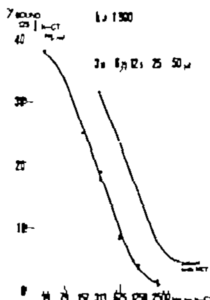


Fig. 1. Linear range of standard curve for serum h-CT using anti-h-CT-serum K5 at final dilution of 1:900 and synthetic h-CT (Ciba) as reference standard. The dilution curve for serum from a patient with MCT is parallel with the standard curve.

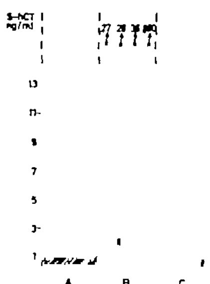


Fig. 2. Serum h-CT levels in 115 healthy controls (shaded reference range) (*A*), before surgery in patients with verified MCT (*B*) and in 9 patients thyroidectomized for thyroid neoplasms other than MCT (*C*). × = actual measured concentration. o = concentration below detection limit (~0...-0.4 ng/ml).

ant. The following injections were made subcutaneously. Eight injections giving totally 7 mg of a 70% extract per animal during eight month produced antibodies to h-CT in one rabbit and two guinea pigs. The antiserum from rabbit K5 was of sufficient specificity for use in the immunoassay and all data in this paper are based on this antiserum.

Iodination mixture

Phosphate buffer 0.05 M pH 7.4 20 μl. Na ^{125}I, 0.5 mCi 5–10 μl. *h-CT in phosphate buffer 0.1 M pH* 7.4 ...5 μg in 5 μl chloramine T in phosphate buffer 0.05 M pH 7.4 20 μg in 20 μl. Oxidation time was 10 sec terminated by the addition of Na-metabisulphite in *phosphate buffer 0.05 M pH 7.4 40 μg in 20 μl.*

The iodination mixture was transferred to a 1 cm 14 cm column of Amberlite CG-400 converted to the acetate state. The column had been equilibrated with and was *eluted with sodium acetate buffer 0.05 M* pH 5.0. Fractions of 1 ml were collected and the active peak (separated from the damaged ^{125}I-h-CT and unreacted Na-^{125}I) usually emerged in tubes 5–10. The *labelled antigen was stored at −20°C and could be used* for at least one month.

Radioimmunoassay incubation mixture

Standard h-CT 0–.5 ng/ml, 0–100 μl phosphate buffer 0.1 M pH 7.4 0–100 μl unknown serum 0–200 μl diluent 10% serum from the h-CT-free patient 0–200 μl Trasylol® (Bayer) 900 KIE 50 μl, antiserum 1:90, 50 μl *h-CT* ^{125}I 100 μl total volume 500 μl.

Antiserum K5 was diluted with NaCl 0.14 M containing merthiolate, 1:10000 /v. The final dilution of antiserum K5 was 1:900. *h-CT* ^{125}I and the standard *h-CT were diluted in phosphate buffer* 0.1 M, with human serum albumin (Sigma), 0.25% and neomycin sulphate (Sigma), 0.02%. The incubation tubes were mixed and left at 4°C for three days.

Separation of bound and free hormone

Separation of bound and free hormones was accomplished by the dextran-coated charcoal method (6). Norit A charcoal (Sigma), 8 g, was suspended in 200 ml distilled H_2O and allowed to settle for 30 min. The water was sucked off until 40 ml remained. Dextran-80 (Pharmacia), 500 mg, was added to the charcoal and mixed vigorously. Phosphate buffer 0.1 M, pH 7.4 with human serum albumin 0.01% and neomycin sulphate, 0.01% was added up to 200 ml. The suspension was *mixed for 1 hour. Of the dextran-coated charcoal* 0.5 ml were added to each tube and mixed in Vortex mixer for ~3 min. The tubes were immediately centrifuged in cold centrifuge for 6 min at 900 *g*. The supernatant was sucked off and the dextran-charcoal residue containing the free ^{125}I-h-CT was counted in a γ-spectrometer. For each tube control without antiserum K5 was incubated to correct for the incubation damage to ^{125}*I-h-CT and for non-specific inhibition of* the immune reaction by different sera. One control serum with normal and one with known high h-CT level were included in each set of incubations.

RESULTS

Properties of the radioimmunoassay for human calcitonin in serum

The best anti-h-CT serum came from rabbit K5. When this antiserum was used at a final concentra-

Table I. *Effects of high concentrations of human and bovine hormones on the assayed h-CT levels*

Hormone	Added amount (ng/ml)	Highest value in normal serum (ng/ml)	Assayed levels of h-CT
h-ACTH[a]	1 000	0.1	Not measurable
h-PTH[b]	335	52	Not measurable
b-PTH[c]	1 000		Not measurable
h-STH[d]	50	10	Not measurable

Synthetic peptide (Ciba).
Our local h-PTH preparation purified from adenomas.
Highly purified (1 500 U/mg, Wilson Co.).
Highly purified, gift by C. A. Gemzell.

tion of 1:900 during 3 days of incubation usually about 25% of added ^{125}I-h-CT was bound to specific antibodies at zero concentration of calcitonin standard.

Specificity

The anti-h-CT serum K5 contained antibodies against h-CT. This is shown by Fig. 1, where a dilution curve of serum from one patient with MCT runs parallel with the linear part of the standard curve. Various concentrations of serum from the patient without functioning thyroid and parathyroid glands did not influence the reaction between synthetic h-CT and its antiserum. A representative standard curve had a linear range between 80 and 1 250 pg.

No cross reactions occurred when large amounts of human parathyroid hormone (h-PTH), bovine parathyroid hormone (b-PTH), human adrenocorticotropin (h-ACTH) and human somatotropin (h-STH) were added (Table I).

Reproducibility

The same samples of sera and plasma from one patient with MCT were assayed in 5–6 sets of incubations (Table II). The coefficients of variation were 12% in serum and 18% in plasma.

Accuracy

Recovery experiments were done with synthetic h-CT. When 10 ng of the peptide was added to standard normal serum, 82–90 ... and when 5 ng was added to serum from a patient with MCT (S-h-CT 5.4 ng/ml), the ... 120–130 ...

Sensitivity and normal range

The sensitivity was adequate enough to permit the measurement of the h-CT concentrations in ... µl aliquots of undiluted serum samples. M ± S.D. from 94 adult healthy blood donors w... 0.70±0.34 ng/ml with ... total observed range of 0.4–1.0 ng/ml (Fig. ...). The levels in ... children 1–19 years old did not diverge from this range. Usually the linear range of the standard curve would allow the measurement of h-CT down to 0.40 ng/ml, sometimes down to 0.20 ng/ml.

Clinical value

The h-CT levels in peripheral ... blood ... measured preoperatively in ... patients ... fied MCT.

We have not seen any patient with ... MCT histopathology and normal h-CT ... false negatives have been found. The h-CT levels were elevated in all patients and ... ranged from 1.6 up to more than 800 ng/ml (Fig. ...). Total thyroidectomy had been performed in one of these patients 3 years before the examination, when he was shown to have multiple lymphoglandular metastases. After removal of these the h-CT level was markedly reduced, but still not normal ... finding consistent with the presence of pulmonary metastases. Thus, also metastases of MCT produce immunoreactive h-CT.

We have hitherto seen no instance of false positive results. In all patients with high h-CT levels who have been operated upon, the presence of MCT could be verified by histopathology. Generally we found a good correlation between the h-CT level and clinically estimated tumour mass.

Table II. *Variations in h-CT levels in serum and plasma from one and the same MCT patient assayed in different sets of incubations*

	Incubations (n)	Individual S-h-CT levels (ng/ml)						Mean	σ	Coeff. of variation (%)
Serum	5	3..	..	..9	..5	2.7	3.4	..94	0.36	1..
Plasma	6	3.5	3.1	3.6	..7	2.4	3.5	3.13	0.57	18

Addition of supposedly calcitonin-free serum to normal sera did not influence their normal levels. Low levels were obtained in assays on untreated sera from 7 of 9 patients thyroidectomized for *tumours other than MCT*

DISCUSSION

The present work describes the properties of a radioimmunoassay for h-CT and some of its clinical applications

The most important properties of our antiserum against h-CT are its high specificity and sensitivity. A prerequisite for the use of the assay in studies of calcium metabolism should be the absence of cross reaction for parathyroid hormone. No such cross reaction was observed over a wide range of concentrations of parathyroid or pituitary hormones

*The major drawback of the first radioimmuno*assay for h-CT was the low sensitivity permitting only high levels to be measured (3). Our h-CT assay is sensitive enough to determine high normal and usually low levels of h-CT. We found no difference in serum levels of h-CT between healthy children and adults. Our reference values are intermediate between the lower levels 0 02–0 40 ng/ml of the radioimmunoassay technique of Tashjian et al. (10) and the higher levels 0 17–35 MRC mU/ml equivalent to 0.6–1 7 ng/ml of the bioassay of Gudmundsson et al (5).

The value of a laboratory method depends not only on the accuracy and precision of the technique but to an equal extent also on its sensitivity and specificity as a diagnostic method in the clinical situation. In this investigation there was an absolute correlation between the presence of histologically verified MCT diagnosed in the conventional manner and high h-CT levels i.e no false negative results were obtained. Nor could we register any false positives. Therefore we conclude. A raised serum h-CT value (≥1.6 ng/ml) is a sensitive and specific indication of the presence of MCT

Both primary and metastasizing MCT produce calcitonin. This means that, postoperatively determinations of h-CT can be used to check whether the tumour has been completely removed. A persisting high level of h-CT after surgery should enforce further examinations for metastases

At present we do not know the clinical significance of h-CT levels close to the upper normal *limit. In these cases the h-CT* measurements during a 4-hour calcium infusion are of special interest, as described by Tashjian et al (10) and Melvin et al (7). The results of our own calcium infusion tests in these borderline cases from three kindreds with Sipple s syndrome will be published later

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JAUNDICE IN PREGNANCY

A Follow-up Study of the Series of Women Originally Reported by L. Thorling

II *Present Health of the Women*

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Abstract In 1955 the late L. Thorling reported on 71 women with jaundice in pregnancy because of cholestasis of pregnancy or infectious hepatitis. A follow-up of 61 of these women, now 40–60 years of age and living all over Sweden, was performed in 1970 Six women had died and four were unwilling to participate. The study included an interview following a standardized questionnaire oral cholecystography (unless the anatomical state of the biliary tract was known) and physical and laboratory examinations (44 women). A moderately abnormal bromsulphalein retention not accounted for by known disease was found in 8 women, three of whom had had symptoms compatible with recurrent cholestasis of pregnancy There were few other indications of liver disease and, in general, the women considered themselves to be in good health Evidence of earlier (cholecystectomy) or present (abnormal cholecystography) gallbladder disease was found in 31 of the 61 women investigated The prevalence was higher in women above (67%) than below (47%) 50 years of age Including the deceased women, the minimal prevalence of gallbladder disease among women with recurrent cholestasis of pregnancy was 57% (16 of 28). Three of the 6 deceased women had had severe kidney disease with uraemia, and 12 surviving women had had symptoms of pyelitis and/or nephrolithiasis. Of the latter urinary tract disease was most common among those with non-recurrent jaundice (8 of 25 32%)

A previous paper (9) reported on the total number of pregnancies in a series of women originally described by Thorling in 1955 (22) because of jaundice during pregnancy Summarizing the course of all pregnancies, it appeared that the women had suffered from several entities of disorders the major ones being cholestasis of pregnancy and infectious hepatitis

Most of the women in the Thorling study were reexamined by us in 1970 i.e 15 years after the original report. The aim of this follow-up was to study whether the women suffered from any late sequelae of the episode(s) of impaired liver function. Another object was to investigate whether the women at this time manifested diseases that may have contributed to the development of jaundice during pregnancy The most important finding is a high prevalence of gallbladder disease among the women with a history of cholestasis of pregnancy

MATERIAL AND METHODS

Of the 71 women reported by Thorling (22), 61 took part in the present study Six women had died and four could not be traced or were unwilling to participate.

On the basis of information obtained at interviews, from the Thorling report and from available hospital records the women were separated into three groups with regard to the recurrence of symptoms (jaundice and itching) (9). In this context, itching alone was considered equivalent to itching and jaundice as sign of cholestasis (1).

Group 1 consisted of 26 women with jaundice and/or itching during more than one pregnancy Six of them had their major symptoms during the first trimester (group 1 A) while 16 (group 1 B) had jaundice and/or itching during the later parts of all their pregnancies and 4 (group 1 C) during the later parts of some of them. All the women in group 1 B and all but one in group 1 C showed clinical picture compatible with recurrent cholestasis of pregnancy Recurrent cholestasis with symptoms both early and late in pregnancy also appeared to be the diagnosis in 3 women in group 1 A.

Group 2 comprised 25 women with jaundice during one of several pregnancies, 10 (group 2 A) had negative and 15 (group 2 B) positive thymol turbidity test during their period of jaundice While several women in group 2 A had symptoms compatible with cholestasis of pregnancy all but one in group 2 B were originally diagnosed as hepatitis.

The remaining 10 women (group 3) bore

Table I. *Age, cause of death and autopsy findings of the deceased women*

Group	Case no. in Thorling report	Age at death (y.)	Cause of death	Additional information from autopsy
Recurrent cases				
I A	31	52	Bronchopneumonia	Chronic pyelonephritis with uraemia, biliary tract normal
I B or I C	39	43	Mammary carcinoma	Not performed
	41	48	Cerebral haemorrhage	Polycystic kidney disease with uraemia, cholecystectomy
	44	45	Uraemia	Nephropathy, cholelithiasis, steatosis of the liver
	69	51	Subarachnoid haemorrhage	Not performed
Non-recurrent case				
B	18	31	Accidental	Internal organs normal

child each, except one woman, whose only pregnancy ended in abortion. Details of the course of pregnancies have been published elsewhere (9).

All 61 women were interviewed following a standardized questionnaire. Information was also obtained from hospital records. Although they were living all over Sweden, it was possible to accomplish physical and laboratory examination in 44; the missing ones (indicated in Fig. 1 as "BSP retention unknown") were distributed almost uniformly between the groups. Venous blood samples were analysed for Hb, ESR, alkaline phosphatases (AP), SGPT, bilirubin, creatinine and serum protein (paper electrophoresis). Urine samples were tested for the presence of glucose and protein. Bromsulphalein (BSP) retention tests were performed by measuri g the dye in blood samples taken every 5 min for 50 min after the injection of 5 mg/kg b.wt. The concentration of the dy was plotted ersus time in order check that the slopes decreased in a semilogarithmic

[illegible] women were examined with oral cholecysto- [illegible] less the anatomical state of the biliary tract was already known. A previous negative cholecystography was accepted if perf rmed less than 5 years before the present study.

The deceased women were subgrouped as described above on the basi of data given in the Thorling report and n hospital records. Additional nformation was obtained from autopsy reports and death certificates.

RESULTS

Deceased women

Five of the six deceased women had had jaundice and/or itching during several pregnancies (Table I). One woman (no. 31 in the Thorling report) had been jaundiced in the first trimester of all her pregnancies and she also had had itching during the last trimester in a way similar to three of the women in group I A. The cause of death was pyelonephritis associated with uraemia. Severe kidney disease was also found in two other women with recurrent symptoms. In one woman (no. 44), postmortem changes were too advanced to allow a definite diagnosis, and the other one (no. 41) suffered from polycystic kidney disease with uraemia. One woman was cholecystectomized and the other one had cholecystolithiasis. The liver and kidneys showed no abnormality in the woman with non-recurrent jaundice (group 2 B) who had died accidentally.

Surviving women

The mean interval between last pregnancy with itching or jaundice and the follow-up varied from 10 to 20 years in the different groups (Table II). In ? women it was less than 5 years and in 6 more than 20. The mean age at the follow-up was 43–49 years (three below 40 and one above 60).

The histories

Most of the women had been admitted to hospital on one or several occasions for reasons other than complications of pregnancy or childbirth. Apart from biliary and renal tract disease (see below), the most common causes of admission were comparatively simple disorders requiring surgical or gynaecological therapy.

Severe disease had occurred in 5 women. One (no. 48, group I B) started to show skin manifestations of systemic lupus erythematosus (SLE) 6 years after her last pregnancy. At the time of the follow-up she had cutaneous, cardiac and hepatic manifestations of this disease and was continuously treated with glucocorticoid hormones. Surgery for mammary carcinoma had been performed in one woman (no. 65, group 2 A) 15 years before this re-examination, still with no evidence of metastases.

Table II. *Age at the follow-up study and time between the last pregnancy ith itching nd/or jaund and the follow-up study (period of observation) (mean and rang)*

Group	No of women	Age (y)	Period of observation (y)
Recurrent cases			
1A	6	44 7 (37 50)	16 9 (9–20)
1B	16	47 8 (40–57)	14 4 (2 21)
1C	4	43 0 (35–49)	10_ (3–15)
Non-recurrent cases			
2A	10	48.5 (39–61)	18 2 (13–20)
2B	15	49 1 (47 55)	20 8 (16–22)
One pregnancy			
3	10	47 7 (43–58)	18 3 (16–27)

Severe metabolic disease characterized by hyper lipoproteinaemia type V (5) and diabetes mellitus developed in one woman (no 13 group 2 B) more than 10 years after the pregnancy complicated by jaundice A liver specimen showed marked steatosis she was treated with insulin Two women in group 3 suffered from rheumatoid arthritis (no 21) and congestive heart disease (no 47) The former received continuous treatment with salicylic acid preparations and the latter was prescribed digoxin and furosemide

Two women in group 1 B had had a febrile disease associated with jaundice during childhood One woman in group B who had suffered from infectious hepatitis during pregnancy had a relapse of this disease (diagnosis confirmed by liver biopsy) 7 years later She had been well since

Apart from nos 48 (SLE) 13 (diabetes mellitus) and 47 (congestive heart disease) no woman who was found to have abnormal laboratory tests was on continuous drug therapy or showed signs of acute infection at the time of the study None of the women admitted addiction to drugs or alcohol

Liver disease

The physical examinations revealed a low incidence of abnormal findings compatible with live disease Palmar erythema was observed in two women (no 27 group 1 A and no 26 group 1 B) The li r was palpable at the right costal arcus in one woman (no 52, group 1 C) and 1.5 cm below that border in another (no 8 group 2 B) Serum bilirubin, conjugated and unconjugated was normal in all women investigated as was SGPT except for those two suffering from SLE and congest e h art disease respectively Normal AP alues re so recorded for all except for the woman wit S Hb was more than 12 g/100 ml n all h woman with kidney disease (see bel women had an ESR of more than 20 mm h of them also had an increased BSP tenu

Serum protein electrophoresis showed n γ-globulins in 11 women Four of them al o l pathological BSP retention test (see below) Am the remainder the highest γ-globulin alue (6 g 100 ml) was encountered in the woman w th rheumatoid arthritis. In the others (4 from group B 1 each from groups 2 A and 3) the γ-globul ns were only moderately increased (1 7–2 0 g/100 ml normal value ≤1 6)

BSP retention at 45 min was more than 5% of the 5 min value in 13 women (Fig 1 Table III) The three women in group 1 A with bnormal BSP retention tests (11–17%) were those who had had symptoms (jaundice and/or itching) only in the first trimester of all their pregnancies In recent years they had often had slight to moderate epigastrial pains. Except for the periods of jaundice during pregnancy there was no history of cholestasis as evidenced by acholic faeces or dark-coloured urine The γ-globul ns were increased in two of the women one of whom also had palmar erythema. Cholecystography was normal in two of them and showed cholecystolithiasis in the third N ne permitted a liver biopsy Serum electrophoresis, serum bilirubin SGPT and AP were checked again in these women I J n. 1974 with unchanged results

Apart from the woman with SLE, three women in group 1 B had a somewhat levated BSP

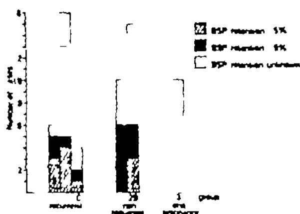


Fig. 1. Outcome of the BSP retention test in relation to itching and jaundice in pregnancy

tion. Two of them complained of occasional epigastrial pains. Liver biopsy specimens obtained from two of these women were normal as evidenced by light microscopy. A slightly abnormal BSP retention test (9%) and a small elevation of the β-globulins were found in one woman in group 1 C (no. 52). She considered herself to be in good health.

The BSP retention test was performed in 19 of the 25 women with non-recurrent jaundice in pregnancy. Three women in group 2 B showed abnormal values. One was the woman (no. 13) with diabetes mellitus, hyperlipoproteinaemia and steatosis of the liver. The second (no. 9) had cholecystolithiasis and moderately elevated γ-globulins. The third suffered from epigastrial pains but had no other signs of liver or biliary tract disease. Two women in group 3 had pathological BSP retention tests. Both were obese, and one also suffered from congestive heart disease.

Biliary tract disease

Oral cholecystography was performed in all but 6 of the non-cholecystectomized women (Fig. 2). The incidence of present (cholecystolithiasis, cholecystitis) or previous (cholecystectomy) gallbladder disease was 53% (31 of 61 women). The prevalence was 65% (13 of 20 women) in groups 1 B and 1 C. Gallbladder disease was more common among the women above (67%) than below (47%) 50 years of age. More than 10% overweight was encountered in 11 women. In the major groups there were no

Table III. *Details about the women with pathological BSP retention tests*

roup	Case no. in Thorling report	BSP (%)	Electrophoretic pattern	State of biliary system	Liver biopsy	Comments
λ	3	17	Normal	Normal cholecystography	–	
	77	11	γ-globulin 1.8	Normal cholecystography	–	Palmar erythema
	3.	12	γ-globulin 2.0	Cholecystolithiasis	–	
1 B	37	9	Normal	Normal cholecystography	–	
	48	6	γ-globulin 3.27	Normal cholecystography	Normal	SLE
	54	8	Normal	Normal cholecystography	Normal	
	61	8	Normal	Normal cholecystography	Normal	
1 C	52	9	β-globulin 1.6	–	–	Hepar palpable at arcus
2 B	9	15	γ-globulin 1.85	Cholecystolithiasis	–	
	13	19	α_1-globulin 1.3 β-globulin 1.7	Cholecystectomized	Steatosis of the liver	Diabetes, hyperlipaemia
	22	9	Normal	Normal cholecystography	Normal	Xanthelasmata
3	7	7	Normal	Normal cholecystography	–	Obesity
	47	8	Normal	Cholecystolithiasis	Normal	Congestive heart disease, obesity

Normal range: BSP ≤5%, α_1-glob. 0.3–0.7 g%, β-glob. 0.5–1.2 g%, γ-glob. 0.7–1.6 g%

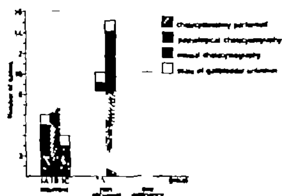


Fig 2 Prevalence of gallbladder disease in relation to itching and jaundice in pregnancy

significant differences between women with or without present or previous gallbladder disease that could be correlated to age parity or body weight (Table IV)

Eighteen women had been cholecystectomized Most of them (13 women) had had the operation after their last pregnancy complicated by itching and/or jaundice (median 8 years range 0–23) Four women in groups 1 B and 1 C had been cholecystectomized in the interval between complicated pregnancies One woman in group 2 B had been operated upon one year before her pregnancy associated with jaundice (diagnosed as infectious hepatitis) All gallstones were of the mixed type

Oral cholecystography showed gallstones in 11 women, I my bile in one and a non-functioning gallbladder in another i.e findings compatible with chronic cholecystitis (3) X ray examination had been performed in all 13 women after the last pregnancy with itching or jaundice

Thorling reported on the result of cholecystography in 17 women performed after a pregnancy with jaundice Of 17 women with a normal cholecystography at that time 5 have since developed gallstones

Urinary tract disease

Urinary tract disease necessitating medical care occurred in 3 women in group 1 B 4 in group A, 4 in group 2 B and 1 in group 3 (*Table V*) Six women (nos 42 48 9 11 22 67) had been without symptoms for several years Of the remaining 6 women 5 had had recurrent infections of the urinary tract for 20–30 years Three of them continuously treated wih antibiotic or chemotherapeutic drugs, were without symptoms while the other two had experienced symptoms during the year preceding this study The sixth woman was found to have hypertension at the follow-up and the subsequent investigation revealed roentgeno-

Table IV *Prevalence of gallbladder disease (GBD) in relation to age parity and body weight (mean and range)*

Group	Prevalence of GBD	Age (y) GBD	Age (y) No known GBD	No. of full-term pregnancies GBD	No. of full-term pregnancies N known GBD	B wt (% of ideal weight)[a] GBD	B wt (% of ideal weight)[a] N known GBD
1 A	2/6	46 5 43 50	43 8 37–49	2.5 2 3	2.5 2–4	98 90 106	107 87–123
1 B	10/16	48 9 42 53	45 8 40–49	2.0 0–4	2 3 2–3	96 83–114	90 84–97
1 C	3/4	41 7 35–49	47	3 7 3–4	3	102 95–105	91
A	6/10	48 2 42–61	49 5 48–51	3 5 2 7	3 0 2–4	98 81–116	100 92–108
2 B	8/15	49 2 42 55	49 1 45–52	6 2–3	2 6 1–4	97 82 112	108 81–133
3	2/10	52.5 47 58	46.5 44–52	1 0 1	0 9 0–1	147 138 160	107 97 132
Total	31/61	48.3	46.8	2 5	1	100	102

Diagnosis obtained by oral cholecystography or operation;no of women with GBD/no. of women in the grou

Calculated as $\frac{\text{weight (kg)}}{\text{length (cm)} - 100} \times 100$.

Table V *Women with present or previous evidence of urinary tract disease*

Group	Case no in Thorling report	History	Present condition	Urinalysis
1 B	36	Recurrent pyelitis and renal stones	No symptoms	Proteinuria
	42	One attack of pyelitis	No symptoms	Not performed
	48	Two attacks of pyelitis	SLE	Normal
2 A	34	Recurrent pyelitis and renal stones	Long-term nitrofurantoin therapy	Normal
	40	One attack of pyelitis	Hypertension One kidney smaller than the other on X ray	Normal
	43	One attack of pyelitis Recurrent cystitis	One kidney slightly smaller than the other on X-ray	Normal
	66	Recurrent pyelitis	Ampicillin therapy for bacteriuria	Normal
B	4	Recurrent cystitis	Long-term sulphonamide therapy	Proteinuria
	9	Renal stones	No symptoms	Normal
	11	Three attacks of pyelitis	No symptoms	Normal
	22	One attack of pyelitis	No ymptoms	Normal
3	67	Renal stones	No symptoms	Proteinuria

logical changes compatible with chronic pyelonephritis Jaundice and/or itching during pregnancy appeared in association with an attack of pyelitis in 4 women (nos 42 and 48 in group 1 B nos 40 and 43 in group A) Serum creatinine was normal in all investigated women as were urine analyses in all but 3 with known urinary tract disease

n to oral cont aceptives

61 women 11 had tried oral contraceptives group 1 A 4 in group 1 B 1 in group 1 C in group ? A 1 in group B and 1 in group 3) The period of treatment varied from 3 days to 18 months (median 4 months) Eight women stopped taking the pills because of side-effects the most common one being a feeling of sickness (5 women) One woman only (group 1 B) got itching which developed on the third day of medication

DISCUSSION

In his report on the present women Thorling distinguished three groups of disorders i.e jaundice in early pregnancy jaundice in late pregnancy and infectious hepatitis (22) The main basis for this distinction was the histories and the outcome of the thymol turbidity tests Summarizing the course of all the pregnancies in these women it appeared that the Thorling diagnoses represented heterogeneous entities of disorders Other pregnancies not included in the Thorling study were either not complicated by symptoms of liver disease or were complicated by pruritus and/or jaundice.

Upon reclassification of the women according to the principles outlined above and described in detail in a previous paper (9) the disorder represented by the women in group 1 B seemed to be homogeneous This condition reappearing during the later parts of all pregnancies was entirely compatible with recurrent cholestasis of pregnancy Half of the women had a strong familial predisposition to develop these symptoms during pregnancy Except for one woman the symptomatology presented by the women in group 1 C (partial recurrence of symptoms) was similar to that of the women in group 1 B

The pregnancies of the women in groups 2 A ("non-hepatitis") and B ("hepatitis) were somewhat heterogeneous with regard to type and course of symptoms This may be because the thymol turbidity test is occasionally abnormal in normal pregnancies (6 1). It has also been suggested that the incidence of positive tests is lower than otherwise when hepatitis is associated with pregnancy (?4) Thus the possibility that some of the women in group A and not all of those in

Table VI. *Prevalence of gallbladde disease (GBD) in the present omen and in two autopsy serie from Malmo General Hospital*

Figures within parentheses denote percentages

	Present series		Autopsy series reported by Sternby		Autopsy series reported by Lindström	
Age (y)	Total no.	GBD (no)	Total no	GBD (no)	Total no	GBD (no)
30-39	3	2	19	2 (11)	1	4 (33)
40-49	40	17 (43)	61	16 (26)	40	6 (15)
50-59	17	11 (65)	105	46 (44)	76	30 (39)
60-69	1	1	126	69 (55)	180	95 (53)
70-79	–		19[illegible]	134 (70)	[illegible]96	186 (63)
80-89	–		175	108 (62)	376	237 (63)
90-99	–		13	12 (92)	77	48 (62)

The study (20) covered 64% of all women who lived in defined area (the city of Malmö) and who died during April 1961 March 1962.
The series (14) included 88% of all persons above 20 years of age who died in the same district in 1969

group 2 B suffered from hepatitis has to be kept in mind

In view of the long interval between the Thorling report and the present study this follow-up offered an unique opportunity to evalute the possible existence of late sequelae of jaundice in pregnancy in one of the largest and earliest series of women with this complication There was some difficulty in persuading the women who now live all over Sweden to participate in both the interview and the physical and chemical examinations But, since the drop-out rat is similar in all subgroups the fact that only 44 of 61 women fulfilled all parts of the study does not seem to invalidate the general conclusions

The histories provided no indication that the women in the large groups (1 B 2 A and 2 B) suffered from severe liver disease and thi mpression was supported by the findings of the physical and laboratory investigations Excluding the woman with SLE the BSP retention test was slightly elevated in only 3 of the women in group 1 B (recurrent cholestasi of pregnancy) The liver biopsy specimens obtained from two of these women were morphologically normal as evidenced by light microscopy Slightly abnormal BSP retention tests were encountered in only two of the women in group B (the woman with diabetes mellitus and hyperl poproteinaemia not included) who probably had had infectious hepatitis One of these women had cholecystolithiasis and elevated γ-globulins Fou other women in this group had normal BSP retention tests but showed some elevation of the serum γ-globulins A similar finding was also recorded in one woman in group 2 A.

Although some of the women mentioned above may have some degree of liver disease the results indicate an excellent prognosis with regard to liver function not only after recurrent cholestasis of pregnancy but also following infectious hepatitis during pregnancy Other studies generally covering much shorter periods of observation, support this conclusion (8 10 1_ 1 22) Residual liver damage following recurrent cholestasis of pregnancy as evidenced by impaired BSP retention tests has been reported for a few women (4 1) Elevated serum γ-globulins have been observed in follow-up studies of subjects w th infectious hepatitis (8) including women w th hepatitis in pregnancy ( ).

Three women of particular interest are those in group 1 A (nos 3 77 and 32) with jaundice and/or itching during the first trimester of all pregnancies ending in childbirth ($n=6$) The symptoms developed n association w th hyperemesis gravidarum, and the women had been exposed to viral hepatitis before their first pregnancy At the time of the follow-up they all experienced epigastral pain and showed evidence of liver dysfunction. The women were unw ll ng to participate in further investigations and the nature of their disease mains obscure

For one part of Sweden the prevalence of gallbladder disease in the general population may be estimated. Most autopsies in the city of Malmö (population 260 000) in the south of Sweden are carried out in one hospital. For women 40–49 and 50–59 years of age the prevalence of gallbladder disease in two autopsy series from this hospital (14, 20) averaged 22 and 42 % respectively (Table VI). The corresponding values in the present investigation were considerably higher—43 and 65 % in the age groups 40–49 and 50–59 years respectively. In the autopsy series the prevalence exceeded 60 % only in women above 70 years of age.

Altogether 13 (65 %) of the 20 women in groups 1 B and 1 C (recurrent cholestasis of pregnancy) suffered from gallbladder disease or had been cholecystectomized because of cholelithiasis. Including the 3 women in group 1 A (nos. 29, 30 and 33) and the deceased women (nos. 31, 43, 48, 45 and 51) who probably also had recurrent cholestasis of pregnancy, the overall minimal prevalence of gallbladder disease in this disorder at the time of the follow-up was 57 % (16 of 28 women).

The prevalence of gallbladder disease is known to increase with age and to be higher in women than in men. Although it is not possible to obtain a figure for the overall prevalence of such disease, it appears to be higher in Sweden than in several other countries (11). The figures recorded r the present women with recurrent cholestasis extraordinarily high and not far from those rted for female Pima Indians in Arizona (19). ese women are reported to have a reduced pool size of the two primary bile acids cholic acid and chenodeoxycholic acid (23), which may or may not be of pathogenetic importance for the formation of gallstones.

Biliary tract disease has been associated with overweight and with parity. However, in the series of Swedish women reported by van der Linden (13) only a weak correlation was observed between gallbladder disease and obesity. Comparisons between the women with and without gallbladder disease in the present study did not reveal any differences in body weight. In the Framingham study (7) the prevalence of gallstone disease was found to be higher in parous than in non-parous women. Among the women in the large groups of the present study, parity was about the same whether they showed gallbladder disease or not. It seems likely that factors other than those mentioned above have contributed to the high prevalence of this disorder.

A number of studies in recent years have demonstrated a disturbed metabolism of steroid hormones in pregnant women with cholestasis of pregnancy (1). The hypothesis that steroid hormone metabolites contribute to the development of impaired bile secretion in this condition gained support from the observation that some women with previous cholestasis of pregnancy react with cholestasis during treatment with oral contraceptives. It has been speculated that these patients have some genetic predisposition to impaired bile secretion not only during pregnancy but also intermittently in the non-pregnant state as a result of a cyclic variation of hormones (1). Such a failure may cause an impaired micelle formation and consequently contribute to the formation of gallstones. This hypothesis is supported by the present study insofar as several of the women with recurrent cholestasis of pregnancy have a strong familial predisposition to develop cholestasis during pregnancy (9). One of the 4 women in group 1 B who were subsequently on oral contraceptives developed itching during therapy.

A high incidence of gallbladder disease was also encountered among the women in groups A (6 of 10) and ? B (8 of 15) who were supposed to have suffered from non-recurrent cholestasis and viral hepatitis. This finding in the latter group raises the question whether these women had pigment stones rather than cholesterol-rich concrements. However, there was no evidence of haemolytic anaemia or liver cirrhosis (15) and all stones removed at operation were of the mixed type.

Next to abnormalities of the liver and the biliary tract, evidence of urinary tract disease was the most common finding at the follow-up. Three of the 6 deceased women had had severe kidney disease with uraemia and 1? surviving women had had symptoms of pyelitis and/or nephrolithiasis. While these symptoms seem to have been sporadic in 6 women, they had probably been recurrent for many years in one woman in group 1 B, four in A and one woman in group B. Including two women from group A, altogether four women had had an attack of pyelitis during the pregnancy studied by Thorling. Jaundice had appeared in association with attacks. A similar connexion between jaundic

in pregnancy and pyelitis has been observed by others (12, 16 17 18)

In his original report on the present women Thorling suggested that jaundice during pregnancy may occasionally be precipitated by extrahepatic infections The present follow-up of the same subjects demonstrates that altogether 8 (32 %) of 25 women with non-recurrent jaundice of pregnancy showed or have shown evidence of urinary tract disease (pyelitis nephrolithiasis or recurrent cystitis) This finding underlines the importance of further investigation into the possible association between jaundice of pregnancy and renal diseases In contrast to women with recurrent cholestasis of pregnancy there was no conclusive evidence of a familial predisposition to develop jaundice and/or pruritus in women with non-recurrent symptoms (9).

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REGIONAL CEREBRAL BLOOD FLOW CEREBRAL METABOLIC RATE OF OXYGEN AND CEREBROSPINAL FLUID ACID–BASE VARIABLES IN PATIENTS WITH ACUTE MENINGITIS AND WITH ACUTE ENCEPHALITIS

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Abstract Regional cerebral blood flow (rCBF) cerebral metabolic rate of oxygen ($CMRO_2$) jugular enous P_{a_2} and cerebrospinal fluid (CSF) acid–base ariables have been studied in fi patient with meningococcal meningitis, fi with pneumococcal meningitis and si with encephalitis. The [133] enon intra-arterial injection method used for the rCBF measurements. The patients were hyperventilating spontaneously The studies ere performed during general nesthesia and artificial ventilation at Pa_{CO_2} adjusted t the level me sured during spontaneous respiration prior to the anesthesia A erage hemispheric CBF was normal and $CMRO_2$ slightly reduced patients with meningococcal meningitis, hereas CBF and $CMRO_2$ were markedly reduced in pneumococcal meningitis and encephalitis. J gular enous P_{a_2} was increased ("luxury perfusion") in meningococcal and pneumococcal meningitis but nor mal i encephalitis. CSF lactic acid was increased and CSF bicarbonat decreased in all meningitis patient nd some ith encephalitis Functional tests of the rCBF were performed by changes of arterial BP and Pa_{CO_2}. Autoregulation (rCBF is normally independent of the BP within wide limit) was frequently impaired, whereas the response t Pa_{CO_2} changes was preserved in most cases (rCBF normally increases with increased Pa_{CO_2}) Focal rCBF abnormalities as disclosed in the control condition w during the functional tests er in frequent n meningitis but frequent in encephalitis The esult suggest that the brain of these patients is ulnerable to factors such as hypertension hypercapnia and hypoxia A more deliberate use of sedatives combined th artificial hyperventilation advocated Epileptic seizures should be counteracted by all available means

In acute meningitis and encephalitis global and sometimes fo l cerebral ymptoms will be present due to more les widespread nflammatory and edematou hanges the tissue It was the aim of the present study to investigate some of the patophysiological mechanisms nvol ed n these processes.

MATERIAL AND METHODS

Sixteen patient abov 15 years of age er studied Fiv had acut meningococcal meningitis fi pneumococcal meningitis, and six acut encephalitis

The patients with meningococcal and pneumococ meningitis had typical moderate to sever ymptom to 5 days duration (verage 3.5 days) nd er studied within the first 4 hours after the diagnosis was established nd antibiotic treatment was started One patient with severe meningococcal meningitis w however first studied after 48 hours. At the time of the study four patient with meningococcal infection were confused and agitated whereas one was awake but lethargic Tw patient with pneumococcal infec tion were confused and agitated. The remaining three were stuporous All fi patients with meningococcal infection recovered Tw of five patients with pneumococcal infection recovered tw survived but with neurological deficit, and one died due to accumulation of secretions in the airways

The patient ith acute encephalitis were studied w thin the first 1 eeks of the disease. The etiology was nknown, although herpes simple was suspected in tw cases based on increasing antibody titer in blood No brai biopsy as performed. At the time of the study tw of the si patients with encephalitis had rather moderat ymptoms, lethargy agitation, confusion and headache The other four patients had sever symptoms mor or les pronounced coma and in one case marked gitation Tw had decerebrate pasticity and three had seizures Artificial respiration was instituted in three cases. The same pattern of abnormalities of the recorded ariable as found in the patients with moderate and with severe encephalitis. All six patient with encephalitis recovered after 3–10 week of hospitalization

The investigations wer carried out under general anesthesia and artificial entilation using time-cycled

Table I *CBF CMRO₂, Jugular P_{O_2} and CSF findings in anesthetized patients with meningitis and encephalitis*

	No. of pats.		Age (y)	Pa_{CO_2} (mmHg)	MABP (mmHg)	Rectal temp. (C°)	Control $CBF_{initial}$ (ml/100 g/min)	Control CBF_{10} (ml/100 g/min)
Meningococcal meningitis	5	Mean	20	3	94	38 6	65	56
		S D		5	3	0.8	18	10
		Range	15–.5	4–38	90– 97	38 1–39 8	43–85	45–69
Pneumococcal meningitis	5	Mean	54	27	86	38.4	35	35
		S D		6	16	0 7	8	5
		Range	40–63	19–33	76–113	37 4–39 0	28–49	29–42
Encephalitis	6	Mean	29	31	82	37 9	29	31
		S D		7	11	0 7	9	10
		Range	17 58	19–38	66– 93	37 0–39 1	17–41	21–46
Normal values in awake man		Mean		40	90	37 0	64	50
		S D		1			9	5

The Pa_{CO_2} values in this Table represent those measured during spontaneous respiration before the measurements. The average values corresponding to the CBF measurements which were performed during artificial ventilation deviated less than 1 mmHg from the average Pa_{CO_2} values given in the Table

respirator (Loosco). The anesthesia was induced with about 200 mg pentobarbital and about 20 mg *d*-tubocurarine chloride It was maintained with oxygen and nitrous oxide at the ratio 1 2 and continuous relaxation was secured with repeated small doses of *d* tubocurarine chloride The ventilation was adjusted in order to maintain the arterial carbon dioxide tension (Pa_{CO_2}) close to that during spontaneous ventilation prior to the anesthesia The Pa_{CO_2} levels during artificial ventilation deviated from those during spontaneous respiration by on an average −0.5 mmHg with a S D of mmHg No major differences were found between three groups of patients.

Blood samples were drawn from two small polyethylene catheters (i. d. 1 mm) placed into the bulb of one of the internal jugular veins and into one of the internal carotid arteries (Seldinger technique) Most often the venous and the arterial catheters were placed on the same side

Cerebrospinal fluid (CSF) was collected anaerobically from the cisterna magna after suboccipital puncture

The regional cerebral blood flow (rCBF) was measured using the ^{133}xenon intra-arterial injection method (9) Briefly this method utilizes rapid injection of ^{133}xenon dissolved in saline into the internal carotid artery via the polyethylene catheter and external registration of the ^{133}xenon clearance curve from the multiple areas of the hemisphere using small scintillation detectors. In the present study 14 scintillation detectors were used Each cyrstal (NaI(Tl)) in the scintillation detectors measured 11 5 mm in diameter and 5 mm in thickness. They were collimated by 43 mm long cylindrical lead tubes with an i. d. of 1 mm The rCBF was calculated from the initial part of the clearance curves ($rCBF_{initial}$) as well as from the clearance curves followed for 10 min ($rCBF_{10}$) The $rCBF_{10}$ and the arteriovenous oxygen difference ($(a\text{-}v)\ O_2$) were used for the calculation of the cerebral metabolic rate of oxygen ($CMRO_2$): $CMRO_2 = CBF_{10} \times (a\text{-}v)\ O_2$

P_{CO_2} P_{O_2} and pH were measured using conventional electrodes (Radiometer) and corrected for the patient's abnormal body temperature (7 14) Oxygen content in arterial and venous blood was calculated from the corresponding values of P_{O_2}, P_{CO_2}, pH Hb, and body temperature and used for the calculation of $(a\text{-}v)\ O_2$. Lactic acid was determined by an enzymatic method (1) Bicarbonate in the CSF was measured by titration using an automatic equipment (Radiometer). Mean arterial blood pressure (MABP) was measured in the internal carotid artery with an electric manometer (in a few patients on the arm by auscultation). The CSF pressure was recorded using the meniscus of CSF in a tube

A control CBF measurement (at the Pa_{CO_2} close to that during spontaneous respiration) was followed by a study during moderate hypertension induced by i. v. infusion of angiotensin (1 mg/l). Then followed in several patients a second control study and thereafter a study during induced hypo- or hypercapnia. The Pa_{CO_2} change was 5–15 mmHg. Most patients with Pa_{CO_2} levels below 25 mmHg during spontaneous respiration were exposed to an increase, and most of those with Pa_{CO_2} above 25 mmHg to a reduction of Pa_{CO_2}. In several patients a subsequent final flow study was performed at the changed level of the Pa_{CO_2} during moderate hypertension induced by a new angiotensin infusion. At the end of the study the catheters were removed. No complications occurred.

(a–v) O_2 (ml/100 g/ min)	$CMRO_2$ (ml/100 g/ min)	J gular Pv_{O_2} (mmHg)	CSF Bicarbonate (mmol/l)	Lactat (mmol/l)	Leucocytes/µl	Pressure (mm H O)
4 1	2.3	49	15 5	7 7	6 514	326
1 5	0.5	4	3 1	1 8	7 16	86
3 6–4 9	1 7 – 8	44–54	10 3–18 3	6 0–10	1 437–18 500	190–400
4 4	1 6	46	16.4	5 8	977	270
1.1	0 6	6	3 3	– 1	837	166
3 3–5.8	1 1–.1	40–53	12.4–20 0	3 9– 8.4	37– 6 072	50–480
5.4	1 7	38	18 4	3	10	130
1 4	0 3	5	3 1	1 1	13	44
3 5–7.3	1.3–.1	33–47	15 6– 1.6	0– 5 0	1 33	80–160
5 6	3.3	38	3 6	1.5	<3	125
	0.5	3	0 9	0 3		

Values from 4 patients, values from 5 patients, alues from 3 patients.

RESULTS

Cerebral metabolism average hemispheric bl d flow in control ondition jugular enous P_{O_2} nd CSF acid–ba e state

The average values, S D and ranges of the moni tored variables n the different groups of patient are given in Table I Rather marked variations were seen in all groups

All patients were *hyperve tilating p ntan ously* the verage Pa_{CO_2} being about 30 mmHg.

The ave age hemispheric blood flow was normal in patients with meningococcal meningitis but re duced by 30–40 % n patients with pneumococcal meningitis and with encephalitis.

For the evaluation of $CMRO_2$ the anesthesia and the body temperature must be taken into con sideration. Based on the other studies of anesthe tized patients ou laboratory ( ) it can be ex pected that the anesthesia used w ll reduce the $CMRO_2$ by about 20 % (to –6 ml/100 g/min) A similar effect of the anesthesia on the CBF may also be expected The nfluence of hyperthermia on the CMRO is to some extent uncertain, in contrast to the influence of hypothermia resulting in the well know $CMRO_2$ reduction Therefore corrections of the $CMRO_2$ due to hyperthermia are not made I the present study the CMRO was reduced in all three groups of patients The reduction was about 30 % in the patients with meningococcal meningitis and was thus not signifi cantly different from what might be expected result of the anesthesia In the patient w th pneumococcal meningitis and with encephalitis the reduction wa about 50 % nd thus much m e marked than that due to the anesthesia.

The jugul venous oxygen t nsion (Pv_{O_2}) w normal n the patients w th encephalitis but s gnifi cantly elevated in those with meningitis The in crease the jugular Pv_{O_2} ndicates that the CBF is higher than that corresponding to the tissue s demand of oxygen—the so-called luxury perfusion yndrome (3)

Studies of the *ebro pinal fluid* revealed in patient with meningitis a metabolic CSF acidosis (ncreased lactate and reduced bicarbonate con centration) Blood lactate concentrations were normal Patients with encephalitis showed only acidosis was only partially compensated by hyper entilation so that the CSF pH values were still below normal In some cases the CSF pH alues were w thin the normal range or slightly above normal Patients with encephalitis showed only minor CSF abnormalities compared w th meningitis patients The presence of leucocytes in the CSF may contribute to the formation of lactic acid (8). This can however not be the only factor since ncreased CSF lactate concentrations were also seen n patients with encephalitis in whom the CSF *pleocytosis was only minimal* The acid–base studies of the cerebrospinal fluid are re ported f rther details in a subsequent com munic tion (5)

We found significantly negativ linear co

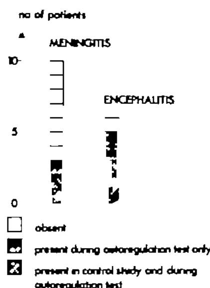


Fig 1 Focal rCBF abnormalities in patients with bacterial meningitis and with encephalitis.

tion between the CSF pH and log CBF ($p<0.01$) and between the CSF pH and the jugular Pv_{O_2} ($p<0.01$). These findings confirm that the brain extracellular fluid pH is a main factor determining CBF (4 16) In addition the findings support the view that the luxury perfusion syndrome is related to acidosis in the brain (3)

abnormalities of the cerebral blood flow q the control condition

cal rCBF abnormalities in the form of hyperemic foci were infrequent in meningitis but frequent in encephalitis. Ischemic foci were only seen in one patient with encephalitis who also had a hyperemic focus (Figs 1 and 2 Table II)

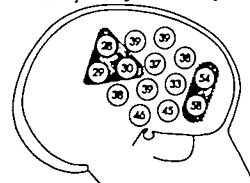
A focus is disclosed by the deviation of the flow values in one or more neighboring channels from the values in the rest of the hemisphere A marked flow deviation in a few channels or a slight deviation in many adjacent channels and the technical quality of the clearance curves including the counting rate (low in peripheral channels) are important factors which have to be taken into consideration in the evaluation of a focal rCBF abnormality Thus no simple definition can be given for a focal abnormality In the present study all focal flow abnormalities encountered in the control condition showed marked deviations of the rCBF of 25% or more from the rest of the hemisphere

Foci with les marked abnormalities were not seen

Regional cerebral bl od fl w findings during functional tests

In addition to the measurements in the control condition (at a Pa_{CO_2} as close as possible to that during spontaneous respiration) the rCBF was measured during one or more functional tests with arterial BP or Pa_{CO_2} changes Normally the rCBF is unchanged during changes of the arterial BP within wide limits—so-called autoregulation. Normally rCBF increases when the Pa_{CO_2} is increased (about 4%/mmHg increase of the Pa_{CO_2})

The rCBF abnormalities encountered are summarized in Table II and consisted of



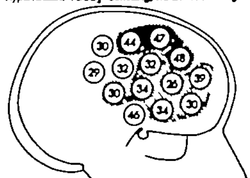


Fig 2 Grossly abnormal regional flow pattern in patient with encephalitis (no. 19) In the control condition (upper figure) a hyperemic focus is present in the frontal region with flow values marked as numbers (rCBF ml/100 g/min) above those in the rest of the hemisphere and an ischemic focus is present in the parieto-occipital region. Induced hypertension (lower figure) disclosed an impairment of the autoregulation in the peripheral frontoparietal region with an increase of the rCBF and a large frontotemporal area with paradoxical flow decrease.

Table II CBF abnormalities in anesthetized patients with meningitis and encephalitis

	Meningococcal meningitis					Pneumococcal meningitis					Encephalitis					
Case no	2	3	8	13	1	4	12	17	18	22	9	10	11	14	19	20
Focal hyperemia at "normocapnia"	−	−	−	−	−	−	−	−	+	−	+	−	−	+	+	−
Focal ischemia at normocapnia"	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−
Globally impaired autoregulation at "normocapnia	+	−	+	−	−	+	−	+	+	+	−	−	−	+	−	−
Focally impaired autoregulation at "normocapnia	−	−	−	−	+	−	−	−	−	+	+		+	−		−
Paradox autoregulation	−	−	−	−	−	−	−	−		−	+	−	+	−		−
Restoration of autoregulation at hypocapnia"													+	−		
Induced loss of autoregulation at hypercapnia"				−			+				+	−	+			−
Globally impaired CO_2 response	−	−	−	−		−	+		−		−	−	+	−		
Focally impaired CO_2 response	−	−	−	−		−	−		−		−	−	+	−		−

+ = present − = absent, "normocapnia" refers t Pa_{CO_2} about that during spontaneou respiration before the rCBF study hypocapnia" to below and "hypercapnia to above.

1 Globally impaired autoregul tion (throughout the whole hemisphere studied) (Fig 3)
2 Focally impaired autoregulation (in circumscript area of the hemisphere stud ed) (Figs. 2 and 4)
3 Focal paradoxical or false autoregulation con sisting of an abnormal focal rCBF decrease in a region of the hemisphere imultaneously to lost autoregulation w th rCBF increase in the other parts of the hemi phere during nduced hyper tension (10 1) (Fig)
4 Globally mpaired response to Pa_{CO_2} changes.
5 Focally impaired response to Pa_{CO_2} change

In some patient the autoregulation was tested at two different levels of the Pa_{CO_2} and some additional abnormal ties were seen

6. Focal restoration of autoregulation during decrease of the Pa_{CO_2}
7 Focally induced loss of autoregulation during increase of the Pa_{CO_2}

All these types of rCBF abnormalities are similar t those seen patient w th poplex y or other types of acute cerebral lesions (10 11)

In some of the patients with global impairment of the autoregulation or the response to Pa_{CO_2} changes the impairment was especially pronounced in focal region. In these instances both focal and global loss of autoregulation or of the response to Pa_{CO_2} changes has bee indicated in Table II

The autoregulation was considered to be globally impaired if the average rCBF increased more than 10% in response to induced hypertension with a MABP ncrease of about 30 mmHg (constancy of the Pa_{CO_2} wa secured) and in most cases with globally lost autoregulation the average rCBF increased about 20% in response to hypertension Globally impaired response to Pa_{CO_2} changes (ncrease or decrease of bout 10 mmHg) wa seen only in few patients in whom the normal re sponse w nearly completely abolished.

Whereas the global abnormalities can easily be haracterized by numerical value It is more difficult to describe the focal abnormalities since among other things, the size of the focus is of importance as discussed above I the patients

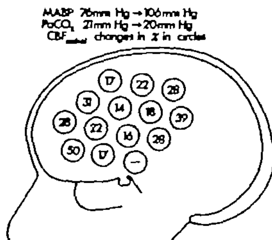


Fig. 3. Globally impaired autoregulation in a patient with pneumococcal meningitis (no. 4).

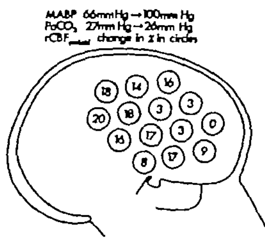


Fig. 4. Focally impaired autoregulation in a patient with encephalitis (no. 10).

with focal loss of autoregulation the focal flow increase during hypertension ranged between 9 and 38 %. Focal loss of the response to Pa_{CO_2} was only seen in one patient with encephalitis who had grossly abnormal rCBF with marked heterogeneity of the flow responses.

Impairment of autoregulation was a much more common finding than impairment of the response to Pa_{CO_2} changes (Table II). Such a stage with dissociated vasoparalysis has previously been observed outside the focal lesion in patients with tracranial tumors and with cerebral apoplexy ...pocapnia could restore the autoregulation (13). ...the present study the dissociated vasoparalysis could in some instances be restored by decreasing the Pa_{CO_2} or could be provoked by increasing the Pa_{CO_2} (Table II).

Focal abnormalities of the rCBF during the functional tests, as also focal abnormalities in the control condition, were more frequent among patients with encephalitis than with meningitis (Table II, Fig. 1).

DISCUSSION

Shapiro and Eisenberg (15) have used the nitrous oxide inhalation technique to study the CBF and metabolism in five patients with St. Louis encephalitis (4 conscious, 1 comatose). This method measures the average CBF. All their patients had a normal CBF but the $CMRO_2$ showed variations on both side of the normal and was especially low in the comatose patient in whom the (A-V) O_2 also was reduced. In this patient luxury perfusion was thus present. These authors' results differ from those in the present study where CBF and $CMRO_2$ decreases were characteristic findings. These differences are probably due to differences in the nature of the infectious process in the various forms of encephalitis, as the methods used for the CBF measurement both by Shapiro and Eisenberg and ourselves are highly reliable and give comparable results.

In the present study the [133]xenon intra-arterial injection method was used for the flow measurements. This method renders determination of the rCBF possible, as each external scintillation detector measures the flow ([133]xenon clearance) from a block of cerebral tissue represented by a truncated cone throughout the hemisphere. The results demonstrated that focal rCBF abnormalities were common in encephalitis but uncommon in meningitis. This difference may be related to pathological changes. In encephalitis the inflammatory processes primarily affecting the brain may have a focal and not only a global character, as is well known, for example, in several cases of herpes simplex encephalitis. On the other hand in bacterial meningitis the infectious process is in most cases rather diffuse throughout the subarachnoidal space and may invade the adjacent brain uniformly. Therefore using the [133]xenon intra-arterial injection method one will get a rather uniform flow pattern over the hemisphere although differences may well occur between more superficial structures in the hemisphere (in the truncated cones measured from) and deeper struc-

tures at a greater distance from the subarachnoidal space

From a pathophysiological point of view the present study indicates that in patients w th acute meningitis or encephalitis the brain is highly ulnerable Factors such as hypertension hyper capnia and hypoxia may even if moderate pro- oke an aggravation of the patient s condition.

Impairment of cerebral autoregulation (vasoparalysis) implies that CBF ncreases when the arterial BP is raised This means that the arteriolar BP is transmitted to the capillaries thus increasing the risk of brain edema during hypertension Episodes of hypertension may well occur during confusion and agitation. It would therefore appear rational to avoid peaks of hypertension by the use of seda tive If depression of respiration cannot be avoided sedation must be combined w th artificial entilation as discussed in the following.

Hypercapnia leads t an aggravation of an intra cerebral acidosis This is because the pH in the brain depends upon the ratio between HCO_3 and CO and because CO_2 crosses the blood brain barrier rapidly whereas the passage of HCO is impeded CBF is mainly determined by the pH of the brain extracellular fluid Lowering of pH causes cerebral vasodilation and vice versa (4 16 17) Hypercapnia thus increases CBF and may also induce loss of autoregulation. In addition hyper capnia increases the intracranial pressure I one patient (no) with an intracranial pressure of 400 mm water in the control cond tion at Pa_{CO_2} of 35 mmHg, the increase of the Pa_{CO_2} to 33 mmHg resulted rise of the MABP from 9? to 140 mmHg (Cushing effect?) The Pa_{CO_2} was immediately lowered agai Thi case illustrates that even moderate Pa_{CO_2} increases to levels well below the normal value of 40 mmHg may cause dangerous effects on the brain The diseased patient may become exhausted due to continuous igorous hyperventilation and/or marked agitation Under such circumstances as well as afte deliberate use of sedatives a ncreasing Pa_{CO_2} may indicate that respir tion should be assisted nd Pa_{CO_2} kept at the hypocapnic le el show by the patient before exhaustion

Hypoxia leads to ncreased production of lactic acid within the brai Thus hypoxia also aggravates an i tracerebral acidosis nd consequently enhances a luxury perfusion with risk of brain edema.

Epileptic seizures frequently accompany acute meningitis and encephalitis. The epileptic seizure in olves hypertension hypercapnia, hypoxia and increased cerebral metabolism (2 6) All four fac tors may be dangerous to the vulnerable brain in these patient We would therefore propose effec ti e pre ention of repetiti e seizures. If neces sary heavy doses of barbiturates should be combined w th artificial ventilation

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ENZYME ACTIVITIES AND GLYCOGEN CONCENTRATION IN SKELETAL MUSCLE IN ALCOHOLISM

The Effect of Abstinence and Physical Conditioning

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Abstract Muscle metabolism of chronic alcoholics has been studied using muscle biopsy technique immediately after drinking period, after 6–7 days abstinence and after one month physical conditioning. The activities of CPK, HK, LDH MDH and SDH were significantly decreased in musculus vastus lateralis for 1 days after an alcoholic debauch. The enzyme activities of the alcoholics, who either had been abstinent for 6–7 days or in addition conditioned for one month did not differ from those of the controls. The concentration of muscle glycogen was at the same level in both groups, but in bicycle ergometer work of an equal relative intensity the alcoholics used more glycogen than the control subjects. The results suggest that the energy metabolism of skeletal muscle is disturbed in an acute stage of chronic alcoholism as judged from the activities of some key enzymes of energy-yielding metabolic pathways. Enzyme activities are normalized during the first week of abstinence, but the consumption of glycogen in physical work still remains larger than in normal muscles after one month's conditioning due to difference in physical fitness.

It is well known that muscle metabolism of alcoholics is disturbed (7) but direct evidence has been obtained only recently when muscle biopsy techniques and microanalytical methods were adapted for this purpose In an acute alcoholic myopathy the serum concentrations of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) are increased (7 11). In muscle of acutely intoxicated alcoholics the concentration of LDH 5 which is the predominant LDH isoenzyme in normal human skeletal muscle is decreased (9) According to Nygren (8) this change is however unspecific and may also occur in connection with other acute diseases The activities of other enzymes of the energy-yielding metabolic pathways and the utilization of substrates in the muscles of alcoholics are not yet known accurately

The purpose of the present investigation was to study some parameters of energy metabolism in skeletal muscle of chronic alcoholics The activities of CPK hexokinase (HK) LDH malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) were measured 1– days after a drinking period after 6–7 days abstinence and after one month's physical conditioning In addition the consumption of muscle glycogen during physical exercise was examined.

MATERIAL AND METHODS

Subjects and experimental arrangements. Two experiments were performed. In experiment I the examinations were made on ten voluntary 4–53-year-old chronic male alcoholics. The muscle biopsies and the ergometer tests were performed after 6–7 days abstinence and after 1 month physical conditioning. The control group consisted of 16 habitually fit men, aged 1 3 years (Table I).

The physical conditioning of the alcoholics was started after the first examination and consisted of daily training of about 2 hours including jogging volley ball, swimming and gymnastics, varying according to the initial physical fitness of the subjects. All physical working capacity (PWC) measurements were performed under controlled condition between 9 and 11 a.m after light breakfast. The subjects avoided vigorous physical activity before the test The muscle biopsies were taken both at rest and immediately after the PWC_{max} test, which consisted of 3 different work loads on bicycle ergometer during 12 min The work loads were estimated be equal for every subject and control in relation to

Table I *Physical fitness enzyme activities initial glycogen concentration and consumption in skeletal muscle of conditioned alcoholics and controls*
I=before II=afte conditioning

	Alcoholics				Controls		p	
	I		II					
	n	Mean±S E.M	*n*	Mean±S E.M		Mean±S E.M	I–II	Alc I-contr
PWC_{150} (kpm/min)	10	810± 68	10	995± 70	16	1 164± 42	<.01	<.001
CPK (U/mg protein)	4	11.66±0 18	4	11 49±2.16	4	10.89±1.36	n.s.	n.s
HK (mU/mg protein)	4	3 7?±0.58		4 14±1.25	4	3.81±0 76	n.s.	n s.
LDH (mU/mg protein)	4	618± 155	4	633± ?62	4	570± 70	n s.	n.s.
MDH (mU/mg protein)	4	938± 78	4	9?7± 45	4	1 068± 48	n	n s.
SDH (mU/mg protein)	4	..20±0.33	4	1 77±0.30	4	1.88±0 16	n.s.	n.
Glycogen[a]	9	*14.20±1.27*	9	*12.20±1..1*	4	*11 7?±0 48*	n.s.	n.s.
Glycogen decrease during exercise[b]	9	3 64±0 79	5	3 40±0 40	24	1 78±0 25	n.s.	< 05

[a] g/kg muscle wet weight. [b] mg/kg muscle wet weight related to work and body weight (kpm/kg)

weight and physical fitness. The enzyme activities and glycogen concentration were measured from every muscle specimen

In experiment II six voluntary 26–47 year-old chronic male alcoholics were examined 1– day after an acute drinking bout. They were selected from the Alcoholic Consulting Center immediately after their admission. Six sedentary healthy men of corresponding ages served as controls. The muscle biopsies were taken only at rest and were used for the analysis of the enzyme activities The experiments were arranged so that the analyses of the muscle specimens of one alcoholic and his control could be performed simultaneously

Muscle biopsies The muscle specimen (10–50 mg) taken from the distal part of m vastus lateralis with needle (Stille Werner) The specimen was immediately freed from blood and connective tissue and ided into two pieces which were then weighed with an automatic balance (Mettler H20T) One piece was homogenized in 0 75 ml ice-cold tris-HCl buffer (pH 7 5) *for enzyme analyses The other piece was deproteinized* in 0 5 ml 3 M HClO for subsequent glycogen determination

Enzymes The muscle homogenate was centrifuged at +4°C for 10 min at 3 000 rpm and the supernatant was used for the determinations. CPK was determined with Biochemica Test Combination (Boehringer/Mannheim) HK according to Silberberg et al (13) LDH according to Kornberg (5) MDH according to Ochoa (10) and SDH according to Earl and Korner (1) The SDH activity possibly represent only a part of the total activity because of the insufficient homogenization The enzyme activities were referred t supernatant protein which *was analysed by a modified method of Lowry et al (6)* Somewhat different method were used the two experiments, e.g. in regard to the interval between the withdrawal of the muscle specimen and the enzyme analyses. Therefore the enzyme activity levels of the two experiments are not comparable The methodological variation in duplicate analyses was les than ±5%

Glycog *For glycogen determination the sample was* neutralized with 2 M KHCO (to obtain a final pH of 7.8–8.5) and centrifuged for 10 min at 3 000 rpm. The sediment was then hydrolysed in 1 ml 1 M HCl at 100°C for hours. From the hydrolysate glycogen w s determined as glycosyl unit by the o-t luidine method described by Hyvärinen and Nikkilä (4)

All spectrophotometrical measurements were performed with Unicam SP 1800 Spectrophotometer

Statistical methods Standard procedures were used to calculate means and S E.M The significances of the differences between the means were calculated with Student s *t*-test or its modification for paired variables (3)

RESULTS

The enzyme levels of the chronic alcoholics who had been abstinent for 6–7 days (experiment I) did not differ from those of the controls Neither did one month s physical conditioning change these enzyme values (Table I)

The physical fitness of the alcoholics was significantly lower than that of the controls ($p<0.001$) measured with the PWC_{150} test both before and after the physical conditioning PWC was however significantly improved during the conditioning (Table I)

The basal muscle glycogen levels of the alcoholics and the controls were about the same but the muscle glycogen concentration during physical exercise was decreased more ($p<0.05$) in the alcoholics The conditioning did not diminish the difference in the efficiency of physical work between the two groups as judged from the utilization of muscle glycogen (Table I)

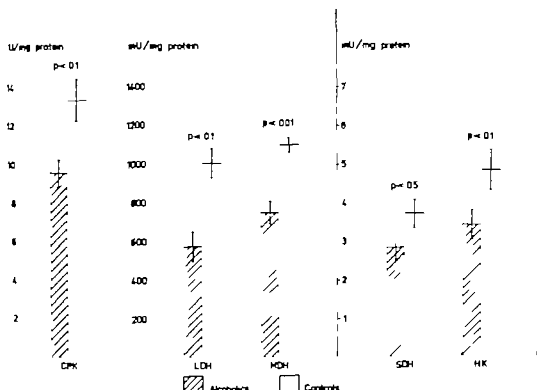


Fig. 1. Muscular enzyme activities (mean ±S.E.M.) of the chronic alcoholics 1 days after a drinking bout and of the controls ($n=6$).

The results of experiment I prompted us to analyse the enzyme activities immediately after an acute drinking bout. In this experiment (Fig. 1) the activities of the measured enzymes were systematically lower in the alcoholics in every six pairs of subjects ($p<0.05$–0.001).

DISCUSSION

The results of the present investigation showed that the activities of the measured enzymes of energy metabolism are decreased in the muscles of chronic alcoholics immediately after an alcoholic debauch (Fig. 1). It has been suggested that the permeability of the cell membrane is increased in damaged muscle cells following an alcoholic debauch and that the muscle cells consequently lose their intracellular enzymes into the extracellular space (7). The increased concentrations of muscular enzymes in serum (7–10) and the decreased enzyme activities in muscles support the suggested increase of the permeability of cell membranes in alcoholics. The disturbances may however be unspecific and also occur in other acute diseases like the reduced LDH-5 content in skeletal muscle found by Nygren (8). Obviously the decreased enzyme activities observed in this study cannot be due to the possible physical inactivity of the alcoholics because there were no significant differences in enzyme values between the alcoholics after 6–7 days abstinence and the respective controls in spite of the significant difference of physical fitness.

Because the enzyme level the muscles of the alcoholics, who had been abstinent for 6–7 days was approximately the same as that of the control subjects (Table 1), t is obvious that one week is enough to normalize the enzymes of the muscle metabolism. Nygren (7) noticed that the serum CPK values returned t normal withi 5–7 days after the alcoholic debauch had terminated. The fast recovery of the activities of metabolic enzymes of the muscle cells is also supported by the

fact that four weeks conditioning did not cause any more changes in the enzyme levels

It has been found that, during exercise trained subjects are able to utilize fat more efficiently than untrained (? 1) The difference in glycogen consumption between the alcoholics and the controls found in this investigation could be due to the better physical fitness of the controls (Table I) or possibly to shifting of the utilization of substrates in the skeletal muscles of the alcoholics towards carbohydrates Because no major differences were observed in the initial glycogen concentrations (Table I) it can be supposed that dietary factors are not responsible for the difference in glycogen consumption.

Whether similar effects of a heavy drinking bout to those observed in the present study of chronic alcoholics can also be demonstrated in normal subjects will be the object for another investigation

ACKNOWLEDGEMENT

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DIAGNOSTIC ERRORS DISCOVERED AT AUTOPSY

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Abstract It has been questioned whether routine autopsies are needed any longer for control and correction of causes of death, specially in lear-cut cases. This question was therefore studied in connection with deaths in department of internal medicine Among 400 consecutive deaths autopsy was performed in 383 (96 %) Causes of death diagnosed before autopsy were compared with those established by the same clinicians after autopsy A main cause of death the clinical diagnosis was thereby confirmed as correct in 57 % of cases, and as erroneous in 30 % I the remaining 13 % it had not been possible to make definite diagnosis ante mortem. Fewer diagnostic errors were encountered among patients below than above 70 years of age There were also fewer errors when linical diagnoses had been considered fairly certain than when estimated only as probable Howeve even in the case of deceased patients below 70 years of age with fairly certain diagnoses, 15 % were revealed t be erroneous at autopsy The main cause of death was circulatory disorder in 67 % of cases, and neoplastic in 17 % All other groups of diseases together accounted for the remaining 16 % of deaths. Clinical diagnoses of neoplasms were more seldom found to be erroneous than diagnoses of other groups of diseases Contributory causes of death were clinically underestimated Of the disorders established as contributory after post-mortem 46 % had been unrecognized before death. It is concluded that autopsies are still needed for control and correction of cause of death, also in lear-cut cases.

In connection with studies of the jurisprudence relating to hospital autopsies in Sweden a need appeared for evaluation of their practical importance (5 6) Earlier it has been stressed repeatedly that frequent post-mortems are necessary for control and correction of causes of death (7 21 22) Lately though it has been questioned whether routine autopsies are of value in this context any longer especially as regards clinically lear-cut cases (8 9) The aim of the present study was to elucidate this question on the basis of deaths occurring in a department of internal medicine

DEFINITIONS

Underlying ause of death is defined by WHO as the disease or injury which initiated the train of morbid events leading directly to death, (26) This cause has in the past also been described as primary cause of death "principal cause of death et and is often equivalent to the expression main diagnosis used in clinical contexts (1) Generally the underlying cause of death is the condition entered on the lowest used line of Part I the death certificate

Contributory caus of death Complications or other than the underlying disease mentioned on the death certificate.

Clinical diagnosis Clinical diagnosis of the underlying cause of death whe nothing else is stated

Final diagnosis Diagnosis of the underlying cause of death as evaluated after autopsy when nothing else is stated

ICD International classification of diseases 8th Revision (26) This classification contains 17 chapters, in the following referred to as main groups of diseases (e.g VII. Diseases of the circulatory system) The main groups are further d vided into sections, here named subgroups (g Cerebrovascular disease), containing varying number of separate categories, diagnoses, each assigned specific code number (g. Subarachnoid haemorrhage ICD 430)

MATERIAL AND METHODS

Serafimerlasarettet is university hospital which at the time of the study served an undefined population in Greater Stockholm. The study covers 400 consecutive deaths (8 %) among 4 895 in-patient admitted to the Medical Department during the period Jan 16, 1970 June 16 1971

Every weekday morning during the survey period the author visited the wards in which patient had died during the previous day( ). Data about the deceased including history and medical findings, were collected In collaboration with the physicians responsible for each particular patient all records were gone through. The cause of death as judged from the clinical point of view as registered as in death certificate.

The cases were divided into three groups according

fact that four weeks conditioning did not cause any more changes in the enzyme levels

It has been found that, during exercise trained subjects are able to utilize fat more efficiently than untrained (1) The difference in glycogen consumption between the alcoholics and the controls found in this investigation could be due to the better physical fitness of the controls (Table I) or possibly to shifting of the utilization of substrates in the skeletal muscles of the alcoholics towards carbohydrates Because no major differences were observed in the initial glycogen concentrations (Table I) it can be supposed that dietary factors are not responsible for the difference in glycogen consumption.

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Table V *Contributory causes of death as evaluated before and after autopsy*

Diseases, main groups ICD	Contributory causes of death as evaluated before autopsy			Contributory causes of death as evaluated after autopsy		
		Disproved at autopsy			Unrecognized clinically	
	Total no.	N	% of total	Total no.	No	% of total
Infective I	7	–	–	11	4	36
Neoplastic II	8	1		22	15	68
Endocrine, metabolic, blood, mental, nervous system, III–VI	79	1	1	87	9	10
Circulatory VII						
Acute myocardial infarction	3	1		27	25	93
Chronic ischaemic heart disease	134	1	16	135	22	16
Other heart diseases	28	5	18	54	31	57
Cerebrovascular disease	31	5	16	53	27	51
Pulmonary embolism	5	3		52	50	96
Other vascular diseases	45	1	2	104	60	58
Respiratory VIII						
Pneumonia	58	21	36	79	42	53
Bronchitis	30	4	13	29	3	10
Digestive IX	15	–	–	55	40	73
Remaining groups, X–XVII	46	9	20	61	4	39
Total	489	7[illegible]	15	769	35[illegible]	46

plicating factors may also be of interest. If they are not discovered or are misjudged, the consequency may be inadequate treatment which might at least worsen the immediate prognosis for the individual.

There are several earlier surveys concerning the total frequency of diagnostic errors as estimated from autopsy materials. These are summarized in Table VI. Due to several circumstances recounted below, however, the results are not easily comparable with those in the present study.

Period studied. Compared to early studies the disease panorama, diagnostic facilities and modes of treatment have changed. Conditions might also differ in different countries.

Errors regarding main or underlying disorder. Nine of the 14 authors referred to in Table VI have not declared whether they considered only inaccuracies regarding the underlying disease or whether they also included more or less important secondary findings in their figures. Where the principal disease was the subject for study, rules for its selection have often been omitted.

Apart from the present study only two others (11, 14) have mentioned that the *clinical diagnosis* was, however, *uncertain or unknown*. In Sweden autopsy is compulsory by law in such cases, which occurred in similar frequency in the two previous and the present surveys (1–15%). In one of them (14), as in the present one, the unknown cases were handled separately. In the remainder all clinical diagnoses, including pure guesses, were included when calculating the total rate of inaccuracies, which should give comparatively higher figures.

Autopsy rate. When autopsy is not performed in almost every case, the material becomes selected. Deceased patients of younger ages, certain disorders and generally "difficult cases" are over-represented (7, 17), which must influence the frequency of inaccurate diagnoses established at post mortem. There is only one other survey (20) with an autopsy rate on the same high level and accordingly with a similar minimum of such selection.

Clinical diagnosis established retrospectively. In all except one (11) of the materials referred to in Table VI, the clinical diagnoses were worked out retrospectively from patient records or autopsy request forms and might therefore be dubious. Pathologists and other investigators not familiar with the clinical picture in a case may also tend to overestimate autopsy findings. To avoid these objections in this study, the evaluations were made by the same clinicians before and after autopsy, which was not done in any of the other studies.

Subject for study and size of material. The aim of most of the major studies was to evaluate the validity of mortality statistics. Inaccuracies due to mistakes made in death certificates or in the central coding were often inseparable from those due to

Table VI *Frequency of diagnostic errors as estimated from autopsy materials a comparison between previous studies and the present one*

Ref no	First mentioned author	Study period or year of publication	Errors regarding main or underlying disorder	Clin. diagnosis unknown or uncertain (% of cases)	Autopsy rate (%)	Clin. diagnosis established retro- or prospectively	Size of material	Total rate of diagnostic errors (%)
15	Karsner	Publ 1919	–	–	20	Retro-	600	68
4	Swartout	1933–37	–	–	40	Retro-	8 080	21
25	Wallgren	1934–39	–	–	7 (low)	Retro-	1 000	49
18	Munck	1940–49	+	–	78	Retro-	1 000	20
10	Gruver	1947–53	–	–	76	Retro-	1 106	6
13	James	1951–52	+	–	24	Retro-	1 889	48
3	Borris	Publ 1952	–	–	50	Retro-	1 078	33
16	Landes	1954–64	–	–	48	Retro-	1 132	48
27	Wilson	1958	–	–	53	Retro-	265	47
14	Justin-Besançon	1958–61	–	15 (excluded)	79	Retro-	1 000	30
11	Heasman	1959	+	12 (not excl)	65	Pro-	9 501	55
20	Otterland	1960	+	–	98	Retro-	377	28
23	Schulz	1967	+	–	18	Retro-	4 652	52
1	Holler	Publ. 1970	–	–	50	Retro-	200	48
	Present study	1970–71	+	13 (excluded)	96	Pro-	383	30

diagnostic errors. Furthermore deaths at all ages and of all causes were included However at least in the more recent *surveys circulatory disorders* predominated followed by neoplasms as in the present material

Bearing all these differences of approach in mind is not surprising to find the total rate of errors losed in the studies reviewed to vary from to 68% the present findings of 30% being among the lowest Beyond *all* doubt anyway it can be concluded that autopsies earlier did and still do reveal a considerable number of errors in clinical diagnoses of patients dying in hospitals.

There is no convincing sign that the rate of errors has diminished over the years although the diseases and types of mistakes have changed Part of the explanation might be that the results of gradually improved diagnostic facilities are cancelled out by a gradually increased proportion of patients of high age among whom diagnostic errors are more frequent

It seems natural and is in accordance with other authors findings (11) that greater difficulties and more inaccuracies exist in diagnoses of elderly patients Their symptoms are often blurred and the presence of multiple diseases further complicates the picture (28)

The clinician s judgement of the certainty of diagnosis turned out to be a good measure of its risk of inaccuracy which was also found by Heasman and Lipworth (11) *Thus when the diagnosis* was estimated as fairly certain It was revealed to be inadequate in 5% of cases but in 45% when considered only probable In the latter category the diagnosis must have been open to question, which seems reassuring The figure of 25% errors in what may be labelled clinically clear-cut cases appears more remarkable Obviously such cases cannot be excluded from autopsy on the grounds that errors in clinically stated causes of death are seldom or ever revealed When no autopsy can be performed though it might be of value for future investigations if certainty of clinical diagnosis was stated on the death certificate. This information together with the age of the patient could be used for a rough estimation of the risk of error of the main diagnosis

Whether clinical diagnoses of the cause of death are certain or uncertain, is there any point in continuing to discover which are erroneous by means of routine autopsies? In the author's view there are many arguments in favour of doing so and some of them will be listed here

For clinicians it has a great postgraduate educational value to have diagnoses checked in each case of death. Disclosing of mistakes stimulates

critical reasoning and increases the willingness to reconsideration, which should in turn improve future patient care With experience from an increasing number of cases more systematic observations can be made which are applicable in current clinical practice Since the disease panorama changes, as do modes of treatment and diagnostics the acquisition of such knowledge must be continuous The experience of clinical diagnostics gained from the present material will be described in a subsequent report (4)

The post-mortem check of clinical diagnoses is important also for reasons other than the post graduate education of doctors that it offers. If autopsies were not performed the central registration of causes of death for individual subjects would be affected This would also be the consequence as regards the Cancer Registry and local records and statistics

Thus without autopsies 7% of the present cases would have been classified as belonging to a wrong main group of diseases in such registers as regards their underlying cause of death Another 7% would have been assigned to an erroneous subgroup of disorders, and 16% to inaccurate ICD numbers within the right subgroups. Almost half of the disorders that contributed to death would not have been recorded at all

However in mortality statistics, the total number of deaths ascribed to a certain cause would not be affected if clinical overdiagnoses and underdiagnoses outweigh one another In the present material this was the case to some extent as regards the underlying cause of death when main groups of diseases were considered The neoplastic group though, was clinically somewhat underestimated whereas there were a few net over diagnoses of most other groups of diseases

Furthermore in the major study of mortality statistics from England and Wales earlier referred to (11) it was concluded that even the total figures would be improved if frequent post-mortems were performed Certainly contributory causes of death would be markedly underestimated without autopsies as judged from the present material

Also to enable studies and follow-ups of disease processes in individual patients it is essential that causes of death are correct i each case. This concerns both the central registration and that in records and statistics of each hospital It is of little help in scientific studies to know that possible errors among investigated non-autopsied subjects are probably outweighed by other errors in other subjects.

For relatives of the deceased the importance of knowing the correct cause of death, at present or in the future must not be underestimated Further more when on rare occasions contagious diseases are disclosed It is of direct practical importance that the relatives are themselves examined

Thus it is concluded that diagnostic errors are still discovered at routine autopsies and that the continuous control and correction of causes of death hereby obtained is important

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CLINICAL DIAGNOSTICS EXPERIENCE FROM 383 AUTOPSIED CASES

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Abstract The aim of the present study was to investigate whether the experience of clinical diagnostics could still further be enriched through routine autopsies. The question was studied by comparing diagnoses made by the same clinicians before and after autopsy in 383 subjects Clinical misinterpretation thereby revealed were further analysed and described Acute myocardial infarction (AMI) was the most common main cause of death. The diagnosis was seldom disproved when clinically considered fairly certain, but the disorder had often been missed ante mortem, especially among patients with known chronic ischaemic heart disease (IHD) Hidden behind this latter label were also cases with valvular lesion or with lung disorders and right heart failure Apart from chronic IHD cerebrovascular diseases were clinically overdiagnosed as main cause of death sometimes recent myocardial infarcts or malignant neoplasm were instead disclosed at autopsy Clinical diagnoses of neoplastic disorders were seldom found to be erroneous, but malignancy should be more often considered clinically In several cases where it had been impossible to establish definite diagnosis on clinical grounds acute abdominal disorders were revealed post mortem. An increased suspicion as regards these diseases seems warranted in obscure cases. The misinterpretations were frequently consequence of our tendency to stick to earlier diagnoses and to overlook the development of new signs and symptoms. The same mechanism might partly explain why disorders contributing to death had often been unrecognized clinically —most frequently pulmonary embolism, AMI, cirrhosis of liver and ulcer of stomach or duodenum It is concluded that the Latin epigram regarding autopsies is still valid Mortui vivos docent let the dead teach the living

In recent years the value of routine autopsies has been questioned (7 9) In a previous paper (5) it was concluded that they were still important for control and correction of causes of death also in what appeared to be clear-cut cases The aim of the present study was to investigate whether in addition, the experience of clinical diagnostics could still further be enriched through such ordinary post-mortem examinations An analysis and description are therefore given of cases where clinical misinterpretations were disclosed at autopsy

MATERIAL AND METHODS

In the Medical Department of Serafimerlasarettet 400 deaths occurred during the period Jan 16 1970 – June 16, 1971 The present survey covers the 383 cases (96%) in which autopsy was performed. Of the deceased patients 203 were men and 180 women Their mean age was 70 years. Detailed descriptions of material and methods as well as definitions are given in previous paper (5).

RESULTS

The results will be presented under different headings according to the main group of diseases to which the clinical diagnosis belonged in the 333 cases in which a diagnosis had been made *Circulatory disorders* predominated as underlying causes of death and will therefore be described first Next in size and order of presentation is the group of *neoplastic disorders* Thereafter the other groups of diseases are described together under the heading *miscellaneous disorders* The 50 cases in which there had been insufficient clinical evidence for a definite cause of death to be established are presented as *cases with clinically unknown causes of death* Finally diagnoses of *contributory causes of death* will be dealt with

Circulatory disorders as underlying cause of death

Clinical diagnoses of circulatory disorders were established in 228 cases. The final diagnoses for 218 of them were still within the same main group

Table I *Circulatory disorders Correlation between clinical and final diagnoses as regards the underlying cause of death*

Clinical diagnoses		Final diagnoses							
		Circulatory disorders							
Disease groups, code nos. ICD	Total no	394–396	410	412	404 421 428	430–438	440–448	450 453	Total no.
Circulatory disorders	228								218
Rheum heart disease 394–396	11	*10*			1				
AMI 410	85	1	*74*	2			2	3	
Chronic IHD 412	56	2	25	*20*	2	1	1		
Other heart disease 404 421–428	10		1	1	*8*				
Cerebrovascular 430–438	61		3	1		*55*			
Arterial 440–48	3	1					*2*		
Venous thrombosis pulm. embolism, 450 453	2							*2*	
Disorders othe than circulatory	105	2	1		3	2		1	9
Total	333	16	104	24	14	58	5	6	227

Figures in italics indicate correct clinical diagnoses.

of diseases In the remaining 10 cases disorders other than circulatory were revealed at autopsy (Table I) Within the circulatory subgroups chronic ischaemic heart disease (IHD) and cerebrovascular diseases were more often over than underdiag nosed clinically The opposite was true for acute myocardial infarction (AMI) rheumatic other heart disease arterial and venous affections

As regards the *rheumatic heart diseases* all clinically diagnosed were confirmed at necropsy In one patient, however a missed active non-rheumatic endocarditis was considered the main cause of death. In four cases rheumatic valvular lesions had clinically been misinterpreted as general arteriosclerosis or IHD (Table I)

In most of the 85 cases in which *AMI* was clinically *considered* to be the underlying cause of death there was no revision after autopsy except for 11 cases In four of the latter the clinical diag nosis had been considered probable and in seven fairly certain

A probable diagnosis of AMI had been made for two middle-aged men who had been admitted as emergency cases with severe central chest pain. One of them had ST elevations consistent with posterior wall infarction. Both patients died in shock a few hours after admission. The one with ECG changes had venous thrombosis and massive pulmonary embolism, the other had an aortic rupture The other probable diagnoses were made on two elderly women with multiple diseases who developed ECG changes suggestive of AMI some days before death. Further evidence of the diagnosis was lacking In one advanced coronary constrictions were proved but no myocardial necrosis In the other there was a small infarct the main disease however turned out to be an *infective* haemorrhagic enterocolitis (Table I)

In four of the seven patients with a clinical diagnosis of AMI considered fairly certain, recent infarct (<8 weeks) was also verified by the pathologist On the basis of other post-mortem findings, however this disease was estimated only to have contributed to death. The underlying disorders in these cases were reassessed as acute haemorrhagic pancreatitis severe aortic stenosis aortic rupture and disseminated lung cancer The re maining three cases with fairly certain diagnosis were women 87 86 and 77 years old They were initially treated in the Coronary Care Unit and the diagnosis of myocardial infarction was made ac cording to conventional criteria, based on history enzyme curves and ECG (18) They all died about 3 weeks after admission. At autopsy none was found to have a recent infarct. One had an old scar and her main disease was considered coronary although she also had a small rectal cancer The other two had massive pulmonary embolism and

Final diagnoses

Disorders other than circulatory

Infective	Neoplastic	Respiratory	Digestive	Total no.
2	6	1	1	10
1	1		1	3
1	3	1		5
	2			2
				96
				106

multiple haemorrhagic infarcts in the lungs. The sources of the emboli could not be definitely established

Chronic IHD was clinically estimated to be the main cause of death of 56 patients In 20 of them this diagnosis was upheld after the post-mortem investigation and in 36 other causes of death were revealed (Table I)

Of the latter as many as 25 were found to have recent myocardial infarction which had not been diagnosed for several reasons. 1) The majority of patients were in a late stage of chronic IHD treated in the hospital for heart failure In some cases the myocardial infarction had occurred on one of the last days before death and had not considerably contributed to the patients already severe symptoms. The diagnosis was therefore not suspected. On other occasions the results of irregular ECGs and enzyme determinations had caused the diagnosis to be ruled out as not very probable 2) Some patients with known chronic IHD died so soon after arrival in the hospital that time did not permit collection of evidence for the diagnosis of AMI 3) On some occasions patients with chronic IHD were admitted for treatment of increasing heart failure. Due to absence of chest pain complaints the correct diagnosis was not suspected.

Chronic IHD was also overdiagnosed at the expense of circulatory disorders other than myocardial infarction. Two patients, above mentioned had rheumatic heart disease Two had myocardial degeneration of non-coronary aetiology One severely deteriorated woman had multiple cerebral softenings and a subarachnoid haemorrhage One man had recent thrombotic occlusions of the renal arteries

In 5 cases with clinical diagnosis of chronic IHD the post-mortem revealed disorders in main groups of diseases other than circulatory Sequelae of pulmonary tuberculosis had caused restriction of lung function and right heart failure in one patient. Chronic bronchitis with severe emphysema had caused similar symptoms in another Two elderly patients treated for heart failure turned out to have pulmonary and pleural metastases the primary site of malignant growth was stomach and rectum, respectively The fifth patient arrived at the emergency ward with central chest pain and IHD was considered a probable diagnosis He died within 3 hours and autopsy disclosed a widespread undifferentiated lung cancer

In 61 patients the clinical diagnosis of a *cerebrovascular disease* was made verified at autopsy in 55 (Table I) The exact type of vascular disease was not specified clinically in 50% of cases The six erroneously diagnosed were among them.

Cases 1–3 had large recent myocardial infarcts which were considered to be the main cause of death One of these patients also had small old softenings in the brain. Another had cerebral infarct, the age of which accorded with the time of her initial moderate symptoms. The myocardial process had appeared later causing deterioration and finally death. In the third patient the brain was not at all affected nor were the carotid arteries.

Case 4 had multiple cerebral emboli apparently dislodged from the heart. Chronic IHD was judged to be the main cause of her death.

Cases 5 and 6 In one man malignant glioma was discovered, and in one woman widespread metastases from breast cancer operated pon many years earlier

There were 15 cases with clinical diagnoses of main cause of death in the remaining circulatory subgroups i.e *othe heart arterial and venous diseases* For 1. of them there was no revision of the diagnosis after autopsy (Table I). For the following three there was Alcoholic heart disease was believed to be the cause of longstanding cardiac arrhythmias in one middleaged man, and an obscure myocardial disorder to be responsible for

Table II *Neoplastic disorders Correlation between clinical and final diagnoses as regards the underlying cause of death*

Clinical diagnoses		Final diagnoses							
		Neoplastic disorders							Disorders other than neoplastic
Disease groups, code nos. ICD	Total no	150–157	162	174	185	200–209	183 186–193	Total no.	
Neoplastic disorders	50							48	
Digestive organs 150–157	8	*8*							
Bronchus or lung, 162	10		*9*						1
Breast, 174	4			*4*					
Prostate 185	6				*5*				1
Lymphatic or blood system, 200–209	*12*					*12*			
Various 183 186–193	6						*6*		
Unspecified site 196–199	4	1	3						
Disorders other than neoplastic	283	4	4	1		1	1	11	272
Total	333	13	16	5	5	13	7	59	274

Figures in italics indicate correct clinical diagnoses.

similar symptoms in another Both turned out to be victims of chronic IHD one had had a recent infarct as well In one woman rheumatic heart disease had been clinically misinterpreted as general arteriosclerosis

In nine subjects earlier believed to have died of other illness circulatory diseases were disclosed post mortem (Table I) One had had a clinical diagnosis of *neoplastic disorder* and eight had had different diagnoses among *miscellaneous disorders*

Neoplastic disorders as underlying cause of death

Clinical diagnoses of malignant disorders were made in 50 cases In 48 of them the final diagnoses still belonged to this group of diseases (Table II) The diagnoses of these subjects also remained within the same subgroup except in 4 cases in whom the primary site of malignancy had been unknown before death In three of the latter it turned out to be in the bronchus and in the fourth in the large intestine

Table III *Miscellaneous disorders Correlation between clinical and final diagnoses as regards the underlying cause of death*

Clinical diagnose		Final diagnoses								
		Miscellaneous disorders								
Disease groups code nos. ICD	Total no.	1–136	40–279	280–389	460–519	520–577	580–629	710–796	800–	Total no.
Miscellaneous disorder	55									42
Infective 1–136	5	*1*					1			
Endocrine metabolic 40–279	4		*4*							
Blood, mental, CNS 280–389	8	1		*5*						
Respiratory 460–519	8				*5*					
Digestive 520–577	14					*12*				
Genitourinary 580–629	7						*6*			
Connective tissue, symptoms 710–796	3						1	*1*		
Violent causes, 800–	6								*5*	
Circulatory disorders	228	2			1	1				4
Neoplastic disorders	50					1				1
Total	333	4	4	5	6	14	8	1	5	47

Figures in italics indicate correct clinical diagnoses.

In two of the eight subjects with malignant neoplasms of digestive organs the tumour had clinically not been correctly located within the gastrointestinal tract. Instead of the pancreas the gallbladder was the primary site in one case In the other case the growth had started in the oesophagus and not in the stomach as was believed

The two deceased patients in whom the cl nical diagnosis of malignant neoplasm was disproved at autopsy had the following histories.

C se 7 A 89-year-old man had urinary trouble. H prostat gland was hard and asymmetric Irregularities suspect of being metastases were seen on X-ray of the skeleton, and di seminated prostate cancer was considered hi main disease H died in bleedings from stomach ulcer At autopsy there were no sign of cancer nor in the subsequently examined microscopic preparations The ulcer was therefore the underlying cause of death.

Cas 8 A 78-year-old woman suffered from fever malaise and dyspnoea. She had pleural effusion enlarged hilar lymph nodes and tumour-like structure in the right upper pulmonary lobe. Autopsy revealed severe mitral stenosis widespread embolism and heart failure as her main disease, instead of the suspected bronchial cancer Inactive tuberculous lymph nodes had caused stenosi of the right upper bronchus and there w an absces peripherally to it.

Of the eleven missed cases with malignant neoplasms (Table II) six had had clinical diagnoses of *circul tory disorders* They were dealt with under the prev ous heading The remaining five cases will be presented according to their clinical diagnosis among *miscellaneous disorders*

Final diagnoses

Circulatory disorders	Neoplastic disorders
8	5
3	
1	1
1	2
	2
1	
1	
1	
18	6
1	48
227	49

Miscell neous disorders as underlying caus of death

Clinical diagnoses in main groups of diseases other than the circulatory or neoplastic had been made in 55 patients (Table III)

Septicaemia was the only infective disease diagnosed clinically as underlying cause of death. This was upheld after autopsy in only one of f ve cases. In three the site of infection and accordingly the underlying disorder was discovered This was pyelonephritis with multiple abscesses in one case and acute endocard tis in two In a fourth case no signs at all of septicaemia were found and blood cultures had been negative Instead venous thromboses and pulmonary emboli revealed at autopsy were considered to ha e caused death and the septic temperature elevations noted before death.

In the other groups of diseases listed in Table III the clinical diagnosis was mostly confirmed at autopsy S x of the cases (nos 9–14) in which it was not will be further described

Case 9 A 99-year-old woman had been thoroughly investigated during several stay in different hospitals, and the diagnosis of sideroblastic anaemia w s established She was treated in this hospital during the last months of her life, during which time she gradually deteriorated with bouts of fever Several antibiotics and 4-week course of antituberculous treatment were tried without any effect. Her main disease was therefore considered to be the above complicated by intercurrent infections. Post-mortem examination however revealed miliary tuberculous lesion in the spleen, li er and bone marrow

Case 10 A middle-aged man was treated for Coombs-positive severe haemolytic anaemia of unknow aetiology H died in haemolytic crisis six days after admission. A reticulum cell sarcoma in mediastinal lymph nodes, spleen and liver was discovered t autopsy

Case 11–12 Two heavy smokers with chronic bronchitis admitted for impaired lung function, turned out to have disseminated bronchial carcinoma

Case 13–14 I two patients with cirrhosis of the liver primary liver cancer had developed, explaining the deterioration for which they were admitted to the hospital.

In four cases clinical diagnoses of *circulatory dis ease* were revised after autopsy to miscellaneous disorders Two of them had final diagnoses of in fective disorders (infecti e colitis tuberculosis), one of respiratory (chronic bronchitis) and one of digesti e (pancreatitis) These cases were described above was the history of a man in whom prostate cancer was disproved and gastric ulcer

was instead revealed as underlying cause of death (case 7).

Clinically unknown causes of death

In 50 patients (13% of all) there had been insufficient clinical evidence for any definite cause of death to be assigned Twenty-six (52%) of these patients had been hospitalized for less than 72 hours before death. The diagnoses made after autopsy are given in Table IV Circulatory diseases predominated as among deceased patients with established clinical diagnoses Instead of neoplasms, however digestive disorders were the second most common cause of death among these clinically unknown cases

Recent myocardial infarcts were disclosed in 15 cases. None of them had had any previous history of IHD They died either soon after arrival in the hospital or suddenly and unexpectedly while hospitalized for other non-lethal conditions. Therefore it was impossible to make a diagnosis on clinical grounds. Similar circumstances existed in three cases in which dissecting aortic aneurysm was disclosed at autopsy

Of the ten cases in whom digestive diseases were revealed some will be mentioned Two of them, previously healthy were admitted with ascites and a disease history of less than one week. ne died a few hours after arrival and the other within a week of hospitalization Both turned out to have had cirrhosis of the liver the former portal vein thrombosis as well The six cases in which acute abdominal disorders, cholecystitis, pancreatitis or ileus were disclosed at autopsy had very similar histories They were brought to hospital because of general deterioration loss of appetite fatigue malaise and fever None had any remarkable signs on physical examination, nor did they complain of abdominal pain. The conditions remained clinically obscure and the correct diagnoses were never suspected

ble IV *Diagnoses of the underlying cause of death established after autopsy in 50 cases considered unknown before death*

Diseases, code nos. ICD	Total	Hospitalized <72 h
Circulatory disorders	31	17
AMI, 410	15	8
Aortic aneurysm, 441	3	3
Chronic IHD 41	5	2
Venous thrombosis with pulm. embolism, 453	2	–
Other	6	4
Digestive disorders	10	5
Cirrhosis of liver 571	2	1
Choledocholithiasis with cholecystitis, 574	3	1
Acute pancreatitis, 577	2	1
Intestinal adhesions with ileus, 560	1	–
Other	2	
Neoplastic disorders	5	1
Other diseases	4	3
Total	50	26

Contributory causes of death

Contributory causes of death were clinically underestimated Thus of 769 disorders established by clinicians as contributory after autopsy 46% had been overlooked before death The most frequently missed diagnoses were pulmonary embolism, AMI cirrhosis of liver and ulcer of stomach or duodenum of which 96% 93% 74% and 73% respectively were clinically missed

The underlying diseases of patients in whom these contributory causes of death had been overlooked were of all types However all except one of the pulmonary emboli afflicted patients with neoplasms or circulatory diseases

DISCUSSION

In the present study the causes of death were assigned on clinical grounds before autopsy and reevaluated by the same clinicians and the author when post-mortem findings were revealed The diagnoses made before and after autopsy were subsequently compared and the cases were analysed from a clinical point of view The author has not been able to find any studies performed in this way which deal with all causes of death.

On several occasions autopsy results have been used for retrospective studies of one or more clinical diagnoses. Some of these will be referred to in the following, although the results are not strictly comparable owing to the different methods of study applied

The same objection can be raised against comparisons with results from studies on the accuracy of mortality statistics. In such investigations, furthermore the cases are not clinically analysed Some observations may nevertheless be relevant

also from this point of view and are therefore referred to. This specially applies to findings in the major British investigation by Heasman and Lipworth carried out in 1959 (11), the only other study of this kind found in which clinical diagnoses had been made before autopsy

In the Medical Department of Serafimerlasarettet the most common cause of death was AMI The diagnosis was seldom disproved when clinically considered fairly certain. The only misinterpreted disorder disclosed at autopsy under these circumstances was pulmonary embolism. Such misinterpretations have been described earlier (12 17) but seem to be possible even today on rare occasions in spite of coronary care units and defined diagnostic criteria. Also in more obscure cases aortic rupture or aneurysm must be considered Furthermore the risk of failure in recognizing other and sometimes more important diseases in patients with correctly diagnosed myocardial infarctions must not be overlooked.

On the other hand AMI had quite often been missed clinically as reported by other authors (4). This had happened especially often among patients with chronic IHD In epidemiological studies these two closely related entities are often combined into one group which of course neutralizes misinterpretations between the two diagnoses In hospitals however the accuracy of each diagnosis is of practical importance since patients are treated differently according to which diagnosis is assumed

Thus a high degree of suspicion of AMI seems to be warranted in patients with chronic IHD Even in the absence of chest pain complaints infarctions should be looked for regularly and particularly when heart failure develops It cannot be excluded that infarcts in such already severely diseased hearts might present somewhat atypical clinical signs. For practical purposes fixed diagnostic criteria should therefore be applied with care in order not to erroneously rule out the diagnosis. For scientific purposes though fixed definitions are often necessary Liberal admission criteria to coronary care units might also be considered when deterioration occurs in patients with chronic IHD However many of these patients are in such a late stage of disease that any measures would be fruitless.

Chronic IHD was, on the whole often erroneously estimated to be the most important disorder of the patient. Careful reconsideration is therefore necessary before the diagnosis is accepted as the main one Apart from AMI already mentioned valvular lesions were hidden behind the label of chronic IHD as has been noted before (3 19) Furthermore breathlessness pulmonary rales and right heart failure are not rarely explained by mechanisms other than backward failure due to chronic IHD Thus disorders in the lungs like chronic bronchitis sequelae of tuberculosis and cancers must be more often suspected in patients with these symptoms

Cerebrovascular disorders were clinically over diagnosed as reported by some authors (8 11) but not by others (14) Single cases of brain tumour and metastatic growth were instead revealed at autopsy Large AMIs seemed to have caused predominating cerebral symptoms in some elderly patients although no arrhythmias were noted Probably this was due to a reduced blood flow caused by impaired cardiac output, in already arteriosclerotic brain vessels Awareness of this symptomatology which has also been noted in retrospective studies (4), and routine ECGs might disclose the correct aetiology in similar cases

Clinical diagnoses of neoplastic disorders were seldom found to be erroneous On the other hand it appears from this material that malignant disorders should be more often suspected clinically This is in accordance with the findings of Heasman and Lipworth (11) They concluded that especially cancers of the lung, and of gallbladder or bile ducts are frequently unrecognized clinically or misinterpreted as malignancies of other sites Examples of such mistakes occurred also among the present few cases.

Among "miscellaneous clinical diagnoses there were examples of missed disorders that are well known as offering particular diagnostic difficulties. Such were subacute endocarditis (6) miliary tuberculosis (16) and malignant lymphoma restricted to abdomen and mediastinum (15) The difficulty involved in diagnosing these conditions must be re emphasized.

In many cases in which an underlying cause of death could not be settled on clinical grounds, this was simply because death had occurred suddenly and unexpectedly It appears more surprising and also sad that in six cases acute abdominal disorders (choledocholithiasis with cholecystitis ileus pancreatitis) had not been revealed, although none of these patients died suddenly Probably the level of

suspicion as regards acute surgical disorders is low in medical wards Accordingly examinations relevant to the respective diagnoses are not carried out. Two of the six patients were examined also by experienced surgeons who nevertheless did not disclose the correct state of matters Therefore in patients with vague symptoms like loss of appetite malaise fever and general deterioration acute abdominal disorders must be seriously reflected on and searched for since on some occasions they might be cured by means of surgical or other intervention

The great majority of diagnostic errors fell upon patients with one or more clarified diseases The tendency of clinicians to stick to earlier diagnoses and overlook the development of new signs and symptoms has been pointed out before (10) Evidently we need to be further reminded that one disease does not give immunity against all others. On the contrary it might predispose to some like cirrhosis of the liver to primary liver cancer and smoking bronchitis to lung cancer

The tendency to overlook some of several simultaneous diseases in the same patient was apparent also as regards contributory causes of death However the frequently non-diagnosed myocardial infarctions and pulmonary embolism were often terinal events in severely diseased patients Death s expected and further investigatory efforts had ased On other occasions the named complications occurred in patients already suffering from circulatory disorders which caused obvious difficulties in differential diagnostics Furthermore when the development of a lung process was recognized it was too often interpreted as pneumonia (5) Several authors have pointed out that pulmonary embolism is the most frequently missed complication among hospitalized patients (1) Nevertheless a greater attention to this disorder is still needed

Overlooked peptic ulcers might also have been terminal caused by the stress of severe disease More surprisingly even advanced cirrhosis of the liver had escaped clinical recognition both in cases where it was the underlying disorder and in those where it was contributory It has been reported earlier though that symptoms and signs are often scanty (13) This of course increases the risk of their being blamed on other coexisting disorders not noticed at all or not until an agonal stage

It is difficult to evaluate the consequences of diagnostic mistakes for the individuals However the general condition of the patient at the time of inaccurate diagnosis appears to be of the greatest importance Misinterpretations between closely related diseases might be of great significance for a patient in good state On the other hand major diagnostic errors may be unimportant in the case of severely ill patients since further investigations or treatments would be fruitless whatever the diagnosis

In the present study on some occasions it might have been of real benefit for the patient if the correct disease had been revealed (i e miliary tuberculosis acute abdomen malignant lymphoma). Great efforts had often been made to arrive at the truth but without success It is hoped that opportunities for involved physicians to solve similar difficult problems in the future will be improved through the experience gained from these cases

Otherwise the diagnostic errors in this material were made in most cases at an advanced stage of disease Therefore they were of little prognostic importance for the individual patients If it had been possible to disclose the correct diseases (i.e neoplasms endocarditis valvular lesions) at an earlier point the outcome for some subjects might have been affected Experience of diagnoses obtained through autopsies therefore should mainly be useful in the care of patients in earlier stages of disease

Thus it appears evident that routine autopsies still provide a knowledge of clinical diagnostics which is useful in current practice

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COLONY FORMING AND COLONY STIMULATING CELLS IN RELATION TO PROGNOSIS IN LEUKEMIA

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Abstract In order to assess the prognostic significance of colony-forming cells (CFC) and colony-stimulating cells (CSC) peripheral white blood cells from patients with leukemia were cultured in agar Before treatment CFC were found in eight of 12 patients with acute myeloblastic or acute undifferentiated leukemia. The four patients who went into complete remission belonged to this group with CFC initially Four patients without CFC initially died without remission. The CFC persisted during treatment, except in the event of severe drug-induced leukopenia. The colony morphology was of the large normal or the small abnormal type initially in remission the colonies were only of the large normal type. Cells with colony-stimulating ability were found in six of the 12 patients before treatment, there was no change in rule during treatment. These six patients included the four with complete remission. All the six patients without CSC died without remission. Since all the four patients with complete remission but only one of those without had both CFC and CSC initially it seems that this combination may be a favorable prognostic sign in acute leukemia, while the absence of CFC and/or CSC may indicate poor prognosis.

Colony-forming cells (CFC) can be demonstrated both in human bone marrow and peripheral blood using an in vitro culture system with a semisolid supporting medium (2, 12, 13 17 21). The exact nature of the CFC is still not known. However it is believed that it belongs to the myeloid precursor cell population.

Colony formation is dependent on the presence of cells producing the colony-stimulating factor (CSF) The mature granulocyte has for a long time been suggested as the major source of CSF in the peripheral blood (9 20 21) Recently however it has been shown that the CSF-producing cell in the peripheral blood is a mononuclear cell most probably the monocyte (4 6 8).

CFC have also been demonstrated in bone marrow and peripheral blood from patients with acute leukemia (10 17 19 21) Some authors have claimed that peripheral blood cells from patients with acute myeloid leukemia (AML) have a larger than normal colony-forming capacity (17 19) while others have shown that a large number of patients lack CFC in the blood (16)

The presence of colony-stimulating cells, here for simplicity called CSC, has also been demonstrated in the peripheral blood from patients with leukemia. Most investigators have found that the peripheral blood cells of patients with AML fail to induce colony formation (18) or do so only when the patients are in remission (9) Others could not find such a correlation to the clinical state of the patient (15)

It has thus been demonstrated that certain patients with acute leukemia have either CFC CSC or both, while others have none of these cells in their blood. In this work an attempt was made to find out whether the presence of CFC or CSC was of prognostic significance

MATERIAL

Fifteen adult patients with leukemia—ten AML, two acute undifferentiated (AUL), one chronic myelocytic (CML) in blastic phase, one erythroleukemia and one aregenerative anemia which later progressed to erythroleukemia—were investigated (Table I). The patient with aregenerative anemia and later erythroleukemia was investigated in both phases of the disease. She was treated with oxymetholone and prednisolone in combination at the time of sampling, and no therapy with cytotoxic drugs has as yet been instituted The patient with CML was treated with combination of vincristin and prednisolone and irradiation of the spleen. All other patients with acute leukemia were treated with intermittent

Table I *Initial data on 15 untreated leukemic patients including colony-forming and colony-stimulating ability in 10⁶ peripheral white blood cells*

Pat no	Sex	Age (y)	Diagnosis	Total WBC/mm³	Blast cells (%)	Trombocytes/mm	No of colonies/10⁶ cells^c Colony forming ability	Colony-stimulating ability
1	♀	67	AML	56 000	97	10 000	0 0 0	0 0 0
2	♂	58	AML	48 000	40	70 000	0 0 0	0 0 0
3	♂	81	AML	161 000	91	44 000	0 0 0	0 0 0
4	♀	70	AML	22 000	90	52 000	0 0 0	0 3 2
5	♀	72	AML	96 000	84	62 000	8 7 11	0 0 0
6	♂	83	AML	51 000	86	10 000	3 7 5	0 0 0
7	♀	63	AML	5 000	81	69 000	5 3 6	3 2
8	♀	64	AML	2 600	3	67 000	2 1 1	2 2 3
9	♀	52	AML	19 600	85	39 000	19 18 16	6 6 4
10	♀	35	AML	4 500	53	134 000	1 2 2	6 3 3
11	♀	56	AUL	3 500	11	81 000	13 15 12	6 8 4
12	♂	60	AUL	16 400	10	3 000	13 10 14	0 0 0
13	♂	71	CML, blast phase	48 200	45	539 000	>350 >350 >350	4 4 4
14	♀	65	Erythroleukemia	55 300^a	54^b	11 000	0 0 0	4 2 2
15	♀	32	Aregenerative anemia later erythroleukemia	2 000	0	62 000	8 10 8	2 3 4
Healthy controls, mean							10	
Healthy controls, range							1 35	

a Total no of nucleated cells b 39 myeloblasts, 15 erythroblasts. c Results from each of triplicate plates.

courses of the combination rubidomycine+cytosine arabinoside+prednisolone with or without pretreatment with l-asparaginase (11)

At the time of the first sampling for CFC and CSC the ...nts were untreated except for blood transfusions (nos 1 2, 6, 8 10 12 and 14) All the following ...les were drawn during the rest period 2–31 days (median 9) after a course of chemotherapy Such follow up investigations could be performed in 12 of the 15 patients Four patients entered remission and are still alive in their first remission lasting various periods (Table IV) The other patients did not respond to therapy and have succumbed except for patient 15 who is alive but not in remission

Sixteen healthy persons were investigated repeatedly and simultaneously for comparison (37 samples).

METHODS

Blood sampling Venous blood, 5–10 ml, with 20 IU heparin/ml was collected from leukemic patients and healthy donors.

Separation of white blood cell (WBC) from heparinized blood was done as described by Böyum (1). Ten parts of a 33.9% Isopaque solution (Nyegaard, Oslo Norway) were mixed with 20 parts of a 6% Dextran 500 solution (Pharmacia, Uppsala, Sweden). One part of heparinized blood was layered over three parts of the Isopaque-Dextran solution and allowed to stand at room temperature The WBC-rich plasma layer was pipetted off when the RBC mass had passed the interphase between the two layers, which took about 20–30 min

Culture technique The method has been described by Robinson and Pike (21) and Robinson et al (19) in detail and was used without modifications.

Feeder layer The culture medium was a modified preparation of McCoy's 5 A medium to which 15% fetal calf serum had been added The medium was mixed with boiled agar to give final agar concentration of 0.5% WBC obtained by Isopaque-Dextran sedimentation, after being carefully washed, were added to the mixture to a final concentration of 1×10^6 cells/ml Aliquots of 1 ml of this mixture were then pipetted into 35 mm Falcon petri dishes and allowed to gel at room temperature before the overlayer was added

Overlayer Peripheral WBC were added to a mixture of modified McCoy's 5 A medium with 15% fetal calf serum and 0.3% agar to final concentration of 1×10^6 cells/ml One ml was then plated over the feeder layer and allowed to gel at room temperature. All cultures were set up in triplicate and incubated in a humidified incubator at 37°C and 10% CO_2 in air Colonies in the overlayer were counted in an inverted microscope (Olympus, Tokyo) after 14 days of incubation

CFC in the peripheral blood from leukemic patients were studied by plating their WBC over a feeder layer of normal human peripheral WBC. CSC in the peripheral blood of the patients were studied by using leukemic WBC as feeder layer in cultures with normal human peripheral WBC in the overlayer Control cultures of

Table II *Colony-forming and colony-stimulating ability in patients who ould be sampled   quentially during treatme t*

Pat. no	Diagnosis	Sample time after diagnosis (d.)	Total WBC/mm³	Blast cells (%)	No. of colonies/10⁵ cells[c]: Colony-forming ability	Colony stimulating ability
	AML	0	20 000	40	0 0 0	0 0 0
		13	3 000	0	0 0 0	0 0 0
		40	11 000	7	0 0 0	0 0 0
4	AML	0	22 700	90	0 0 0	0 3 2
		35	6 000	98	0 0 0	0 2
		48	1 600	56	0 0 0	1 2 2
5	AML	0	62 000	84	8 7 11	0 0 0
		9	7 700	84	3 2 1	0 0 0
		37	3 000	18	1 2 1	0 0 0
6	AML	0	51 000	86	3 7 5	0 0 0
		9	10 400	91	3 2 1	0 0 0
		37	12 000	94	1 1 1	0 0 0
8	AML	0	2 600	3	2 0 1	2 3
		7	1 200	0	18 16 16	4 3 2
		60	900	0	0 0 0	1 2 1
		120	4 200	0	2 1 2	1 1 1
		150	3 290	0	2 1 2	1 1 1
		215	2 900	0	1 1	1 0 2
9	AML	0	19 600	85	19 18 16	6 6 4
		10	1 800	51	6 4 7	7 5 5
		50	2 700	0	9 7 8	10 9 9
		70	4 100	0	1 1	0 1
		116	4 900	0	0 0 2	1 2 1
		190	3 500	0	1 0 1	Not done
10	AML	0	4 900	53	1 2	6 3 3
		20	900	0	0 0 0	13 18 15
		102	4 500	0	6 5 5	5 3 4
11	AUL	0	3 500	11	13 15 12	6 8 4
		28	680	0	0 0 0	2 1 2
		68	8 800	0	2 1 3	3 1 3
12	AUL	0	16 400	10	13 10 14	0 0 0
		8	2 400	32	238 211 224	7 6 8
		66	1 200	0	6 8 7	0 0 0
13	CML, blast phase	0	48 200	45	>350 >350 >350	4 4 4
		28	163 000	77	192 167 182	7 5 8
14	Erythro-leukemia	0	55 300[a]	39/15[b]	0 0 0	4 2 3
		32	17 500[a]	55/5[b]	0 0 0	27 31 3?
		6[illegible]	65 600[a]	69/8[b]	0 0 0	0 0 0
15	Aregenerative anemia, later erythro-leukemia	0	2 000	0/0	8 10 8	3 4
		46	4 500	0/0	280 300 280	18 16 16
		105	13 900[a]	2/32[b]	8 6 8	Not done
		142	31 500[a]	0/94[b]	0 0 0	0 0 0

Total number of nucleated cells Myeloblasts/erythroblasts. Results from each of triplicate plates.

peripheral WBC from healthy subjects were set up at each experiment using cells from the same healthy individual both in the feeder layer and the overlayer

RESULTS

Colony-forming cells

Eight of the 1 patients with AML or AUL had CFC before treatment No correlation could be found either to total WBC counts or to the per cent blast cells at the time of the sampling (Table I)

Peripheral WBC from healthy controls ga e rise to a mean number of 10 colonies (range 1–35) (Table I). The patient with CML i blastic phase had a markedly increased number of CFC The patient with erythroleukemia did not form colonies and the patient with aregenerative anemia had an initial normal or high colony-forming ability (Table I) which however during the progression of the disease to erythroleukemia went down to 0 (Table II).

Table III. *Morphology of colonies in different stages of the disease*

RM=remission Ll=large loose Lc=large compact, Sc=small compact, Nc=no colonies

Pat. no.	Diagnosis	Clinical state	Colony type
5	AML	Untreated	Lc
		No RM	Sc
		No RM	Sc
6	AML	Untreated	Lc
		No RM	Lc
		No RM	Sc
8	AML	Untreated	Sc
		No RM	Sc
		Partial RM	Nc
		Complete RM	Ll
		Complete RM	Ll
		Complete RM	Ll
9	AML	Untreated	Lc
		No RM	Lc
		Partial RM	Sc
		Complete RM	Sc
		Complete RM	Sc
		Complete RM	Lc
10	AML	Untreated	Sc
		No RM	N
		Complete RM	Ll
11	AUL	Untreated	Ll
		No RM	Nc
		Complete RM	Ll
12	AUL	Untreated	Ll
		No RM	Sc
		No RM	Sc
13	CML, blast phase	Untreated	Ll
		No RM	Ll
15	Aregenerative anemia, later erythro-leukemia	Untreated	Sc
		No RM	Ll
		No RM	Ll and Sc
		No RM	Nc

The colony-forming ability generally persisted during treatment and disappeared only during severe leukopenia induced by cytotoxic drugs (patients 8 and 10 at a WBC count of 900/mm³ and patient 11 at 680/mm³ Table II). All four patients who entered remission belonged to the group with initial colony-forming ability

Colony stimulating ability

Six of the 12 patients with AML or AUL had CSC in their peripheral blood Such activity was also present in both erythroleukemia patients and in the patient with CML in the blastic phase The average number of colonies induced by leukemic cells with colony-stimulating ability was however lower than the number of colonies induced by normal feeder cells (Table I) All patients with AML or AUL who initially demonstrated this activity continued to do so during treatment. Only one patient who lacked stimulating ability initially acquired this ability after treatment. Both patients with erythroleukemia lost their stimulating ability during progression of the disease (Table II) All four patients who entered remission belonged to the group with colony-stimulating ability Only two of the 12 AML or AUL patients with stimulating cells (nos 4 and 7) but all six without these cells died without remission.

Colony morphology (Table III)

The morphology of colonies after 14 days of in vitro incubation could be divided into three groups. large loose large compact and small compact types Peripheral WBC from healthy donors give rise to large colonies, usually of the large loose type but occasionally also of the large compact type. The small compact type colonies were composed of 50–170 cells with an average of 75 cells per colony the large types of 300–1 200 cells with an average of 700 cells per colony In the patient material all types of colonies were represented at the time of the first sampling The AML patients and the patient with aregenerative anemia had colonies of the compact type while the two patients with AUL and the patient with CML in the blast phase had the normal loose type of colonies During treatment the morphology of the colonies remained unchanged or changed to any of the three types. All four patients who went into complete remission developed the large normal type of colonies during remission, three of the four had the large loose type and one the large compact type of colonies, while all three AML or AUL patients who could be sampled over a sufficient period and who did not enter remission had the abnormal small compact type of colonies at the end of their disease.

Colony-forming and colony-stimulating cells in relation to prognosis (Table IV)

The combined presence of CFC and CSC was found in all four patients with AML or AUL who entered remission. Of the remaining patients with these diagnoses only one had the same combination without entering remission A similar combination was however found in the patient with CML in blastic crisis, who died and in a patient with

Table IV *Colony-forming and colony-stimulating cells in untreated patients and the clinical course of the disease*

Pat. no	Diagnosis	Colony forming ability	Colony-stimulating ability	Clinical course: Complete remission time (d.)	Clinical course: Survival
1	AML	−	−	None	Died 8 d. after diagnosis
2	AML	−	−	None	Died 93 d. after diagnosis
3	AML	−	−	None	Died 17 d. after diagnosis
4	AML	−	+	None	Died 100 d. after diagnosis
5	AML	+	−	None	Died 51 d. after diagnosis
6	AML	+	−	None	Died 40 d. after diagnosis
7	AML	+	+	None	Died 70 d. after diagnosis
8	AML	+	+	210	Alive 770 d. after diagnosis
9	AML	+	+	166	Alive 223 d. after diagnosis
10	AML	+	+	205	Alive 256 d. after diagnosis
11	AUL	+	+	58	Alive 124 d. after diagnosis
12	AUL	+	−	None	Died 90 d. after diagnosis
13	CML, blast phase	++	+	None	Died 38 d. after diagnosis
14	Erythroleukemia	−	+	None	Died 89 d. after diagnosis
15	Aregenerative anemia, later erythroleukemia	+	+	None	Alive 173 d. after diagnosis of anemia, 57 d. after diagnosis of leukemia

+ = within normal limits, ++ = more than normal.

aregenerative anemia who later developed erythroleukemia and is alive but not in remission. In the overt leukemic phase of the latter patient the CSC found in the initial stage of the disease had disappeared. It thus appears that an initial combination of CFC and CSC may be a good prognostic sign in patients with AML or AUL.

DISCUSSION

Peripheral WBC from patients with acute leukemia were shown in this study, as in other previous studies (16–19), to be able to form colonies in an in vitro agar culture system. A great variation in the morphological appearance of the colonies was also noted. Both the normal large and the abnormal small types of colonies previously observed in patients with acute leukemia (18) were found. Whether this variation in colony morphology can be explained by the proliferation of different cell clones, normal and leukemic, is still unclear. It has been convincingly shown, using chromosome markers, that leukemic cells are capable of forming colonies in agar (2, 5, 14), but it is still debated whether normal cells as well may form colonies in cell cultures from untreated patients.

During treatment the morphology of the colonies either changed from the original appearance to any of the three patterns: small compact, large compact, large loose, or remained unchanged. Although there was no clear correlation between the type of change and the prognosis, all four patients who entered remission had the normal large type of colonies in this stage of the disease. This observation is in agreement with previously observed normalization of the colony type in remission (14).

Most of the patients in the present study formed colonies before institution of treatment, which is at variance with some other studies (9, 16). It has also been reported that cells from patients with a high number of blast cells give rise to the greatest number of colonies (19). We could not find this type of correlation. During treatment there was generally no change in the colony-forming ability. Only during severe leukopenia was this ability lost. Thus cytotoxic drugs are capable of destroying the CFC in the peripheral blood, although these cells seem to be more resistant to therapy than most other cells. Such therapeutically induced disappearance of CFC occurred in three of the four patients entering remission, and in all these three (nos. 8, 10, 11) large loose colonies reappeared.

In the patient with CML the number of colonies was very much increased, which is in agreement with previous findings by Moore et al. (16). In the blastic state, however, we did not find any apparent decrease in the colony-forming capacity previously noted by the same authors. The patient with preleukemic aregenerative anemia had initially normal colony-forming ability in contrast to some reports of abnormal clones in preleukemia (6, 22).

However after progression to erythroleukemia no colonies were formed and this pattern was similar to that found in the second case of erythroleukemia

None of the six patients in our study who initially lacked cells with colony-stimulating ability entered remission while all four who did so had cells with colony-stimulating activity During treatment there was generally no change in the ability to stimulate colony formation Only one patient with initial inability to stimulate colony formation had a transient change to colony-stimulating activity during treatment

In this study the presence of both CFC and CSC was found in all four patients who entered remission. Only one AML patient with this combination did not enter remission It is not possible from our investigation to state that this is a requirement for remission However it seems intriguing to speculate that the presence of these two cell types found also in normal persons may be of prognostic importance The patient with CML in the blastic phase as well as the patient with aregenerative anemia, both had colony-forming and colony-stimulating ability Here a combination of CFC and CSC was not of prognostic value However the progression of these diseases may have a different pathogenesis than that of ML and AUL.

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DEFECTIVE DIFFERENTIATION OF MEGAKARYOCYTES *IN ACUTE MYELOID LEUKEMIA*

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Abstract A patient with acute myeloid leukemia chromosome analysis, using banding technique revealed an isochromosome 17 in granulopoietic and erythropoietic precursors as well as in polyploid cells, probably megakaryocytes. An abundant number of megakaryocytes in the bone marrow permitted characterization of the ploidy levels of these cells. It is demonstrated that most megakaryocytes were diploid and that polyploidy was not normally developed. The results indicate defective differentiation of the megakaryocytes and/or their precursors analogous to what has previously been demonstrated in the myeloblasts of acute leukemia.

An increasing amount of data indicate that leukemic blast cells have a low proliferative activity and reduced ability to differentiate (9 10) A delayed maturation of these cells has been demonstrated even in the preleukemic phase (6). Krogh Jensen and Killmann (11 12) have presented evidence that erythroblasts may likewise be of leukemic origin in acute myeloid leukemia (AML), and it has been suggested that the proliferative activity of the erythroblasts is decreased (7 17) Less is known about the proliferation and differentiation of megakaryocytes in AML, although thrombocytopoiesis is obviously always affected in acute leukemia.

In the present report of a patient with acute leukemia chromosome studies indicated an abnormally low degree of polyploidy in the megakaryocytes pointing to a reduced differentiation of these cells in line with what has been reported in leukemic blast cells.

CASE REPORT

The patient is a 78-year-old female with increasing fatigue and a tendency to bruising and epistaxis over the past 3–4 months. On admission Hb was 7 1 g/100 ml RBC 2.6 mill., Hct 22.1% MCV 96 μ^3 and MCHC 34 g/100 ml. The WBC count was 700 and a differential count showed 24% neutrophils, 34% lymphocytes, 30% monocytes, 2% eosinophils and 3% basophils. No definite blast cells were seen but 7% of the cells were classified as small megakaryocytes. The thrombocyte count was 75 000 The bone marrow findings are described below (see Results). Serum iron was 130 μg/100 ml and transferrin 260 μg/100 ml. Serum B_{12} 390 pg/ml and folic acid in whole blood was also normal 27 ng/ml. Serum lysozyme was normal and the urine contained no detectable lysozyme. An abdominal X-ray revealed no splenomegaly

During the first two weeks after admission the Hb concentration fell to 5 4 g/100 ml and transfusions were given. The patient has hitherto been followed for 6 months and repeated blood transfusions have been required. WBC have remained low about 1 900–2 000 and the thrombocyte count has fallen to about 25 000. There has been a successive increase in the proportion of blast cells in the bone marrow from 11% to 40%

MATERIAL AND METHODS

The investigations described below were performed immediately after the first admission.

Bone marrow smears were stained with May-Grünwald-Giemsa and a differential count of 1 000 cells was performed. One thousand mitotic cells were examined and the proportion of mitotic figures assigned to megakaryocytes was determined.

Chromosome preparations. A bone marrow aspirate was collected in a heparinized syringe and immediately incubated at 37°C in Basal Medium Eagle containing 0 1 μg/ml Colcemide® After 1 hour the cells were rinsed twice in Hank BSS, transferred to 0 075 M KCl for 20 min as a hypotonic treatment and subsequently fixed in several changes of methanol acetic acid 3 1 Air-dry spreads were prepared and stained with Giemsa according to the method described by Seabright (21) with slight modifications The chromosomes were classified according to criteria given by the Paris Conference 1971 (16).



Fig. 1. Karyotype of a diploid bone marrow cell stained by a G-banding technique showing one chromosome 17 replaced by one isochromosome 17 (arrow).

RESULTS

Th bone marrow smears were very rich in cells with a myeloid erythroid ratio of 3 1 There was a striking predominance of immature granulopoietic cells and 11 0% of the bone marrow cells were assified as myeloblasts In addition an accumula of megakaryocytes 7 8% was noted Most of se cells were immature with a basophilic non granulated cytoplasm Within the erythropoietic pool a considerable number of cells with a megaloblastoid appearance were noted Of the mitotic figures 57.6% were found in granulopoietic precursor cells 39 7% in erythroblasts and 2.5% in megakaryocytes

Chromosome analyses The scanning of 3 000 metaphases for polyploidy resulted in the finding of 7 octaploid and 21 tetraploid cells The chromosomes of 100 diploid and 2 tetraploid cells were counted All the diploid cells had 46 chromosomes and both tetraploid 92 The two tetraploid cells and 35 of the diploid cells were karyotyped All diploid cells analysed showed the same pseudodiploid karyotype one chromosome 17 was replaced by one metacentric marker chromosome of C group size The Giemsa banding pattern of all cells analysed indicated that the metacentric marker was an isochromosome for the long arm of a chromosome 17 (Fig. 1) The two tetraploid cells showed the exact double pseudodiploid karyotype (Fig. 2)

DISCUSSION

Several clinical and laboratory data on the patient are highly suggestive of a malignant hematologic disease Anemia, thrombocytopenia and leukopenia associated with a hypercellular bone marrow dominated by immature granulopoietic cells and with a high proportion of myeloblasts are consistent with the diagnosis of acute leukemia. The constant cytogenetic abnormality including a conspicuous marker chromosome in all bone marrow cells also provided strong evidence in favor of the diagnosis of leukemia There was also an accumulation of immature megakaryocytes in the bone marrow similar to what has previously been described as acute aleukemic megakaryocytic leukemia (1 18) and in a case of refractory anemia highly suggestive of aleukemic AML (2).

Chromosome abnormalities are present in about 50% of acute leukemias (20) and it has been demonstrated that the karyotypic abnormalities may be common to granulopoietic and erythropoietic cells (11 12) The results in our patient confirm that granulopoietic precursors and erythroblasts may have a common chromosome aberration since in

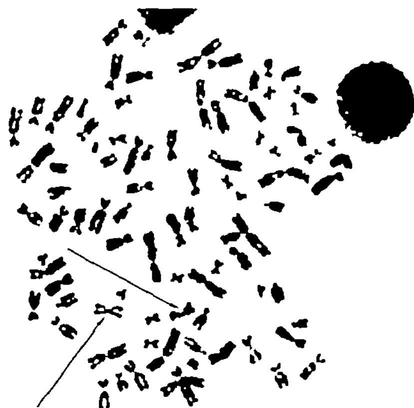


Fig. 2. Metaphase plate of tetraploid bone marrow cell stained by G-banding technique. The two isochromosomes 17 are indicated by arrows.

all bone marrow metaphases analysed by banding technique a marker chromosome—isochromosome 17—was found to replace a normal chromosome 17.

The abundance of megakaryocytes in the bone marrow of the present case offered a relatively unique opportunity to study these cells. It seems well established that cells recognizable as megakaryocytes in the bone marrow are normally polyploid (13) and that 70–90% are cells with 16-ploidy or more (8 15). It has been demonstrated that DNA synthesis and nuclear replication occur only in the most immature megakaryocytes (3 5). In our patient 99 % of the metaphases were diploid and only 0.8% were polyploid. Thus the percentage of polyploid metaphases was only one third of that expected from the analyses of the bone marrow smears. We therefore conclude that a large proportion of the megakaryocytes with mitotic figures were [illegible]x cells. Furthermore [illegible] of 23 polyploid metaphases found in the chromosome preparations were tetraploid cells (85%) and only two octaploid cells were noted. No cells with higher ploidy level were seen. Assuming that the polyploid mitotic figures observed belonged to the most immature megakaryocytes these results are consistent with an abnormally low degree of ploidy in these cells, since Odell and Jackson (13) found 89% with ploidy values higher than 4x in immature megakaryocytes. No metaphase with 16x or more was found in the bone marrow of our patient which further emphasizes that polyploidy was not normally developed in her megakaryocytes.

It has been suggested that individual megakaryocytes may reach full maturity and shed thrombocytes at different ploidy levels (4 14) and that the size of the mature megakaryocyte is related to the degree of polyploidy (14). Mature megakaryocytes with low ploidy values may therefore produce relatively few platelets. The finding of a low degree of polyploidy of the megakaryocytes in raises the question whether at least in part due to a reduced polyploidy. Despite the presence of

penia Saarni (19) found normal or increased numbers of megakaryocytes in the bone marrow of most patients with acute myelomonocytic leukemia. The degree of ploidy of the megakaryocytes in acute leukemic conditions with low platelet counts may therefore deserve further investigation in order to clarify the mechanism causing thrombocytopenia.

Ebbe (4) and Odell and Jackson (14) have proposed models for megakaryocytopoiesis in the rat. The evidence favors the existence of a morphologically unrecognized polyploid precursor pool that feeds the megakaryocytic compartment. The precursor compartment is composed of cells capable of nuclear replication but not of cell division. Assuming that a similar compartment exists in man, the finding of immature megakaryocytes with a low ploidy in our patient may indicate that replication was blocked before the cells had entered the recognizable megakaryocytic compartment.

Our results indicate a common chromosome abnormality in granulopoietic, erythropoietic and megakaryocytic cells, suggesting a clonal origin of all these types of cells in the present case. As mentioned initially, several data argue in favor of a reduced capacity of the myeloblasts to differentiate in AML, and a similar defect is probably ... in the erythroblasts. The finding of an ...rmally low degree of polyploidy in the mega...ryocytes of our patient indicates that the differentiation of megakaryocytes or their precursors may be blocked in a similar way in acute leukemia.

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IRON INTOXICATION IN TWO ADULT PATIENTS

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Abstract Iron intoxication in adults is very rare. Only few cases are reported in the literature. This paper describes two successfully treated adult patients, one without complications and the other with circulatory failure hepatic involvement and hemolysis. The current opinions on pathogenesis and therapy are reviewed.

Acute poisoning with iron compounds is a common problem in clinical pediatrics Some 2000 cases are reported annually from the United States (13) Formerly there was a high incidence of mortality (50%) but since the introduction of desferrioxamine a chelating agent promoting the urinary excretion of iron the prognosis has improved

In adults iron intoxication is an unusual clinical problem and the iron compounds are mostly ingested with the intention of committing suicide Only a few cases have been reported in the literature (4 5 10 13) In the present report two cases of acute iron intoxication one without complications and the other with hepatic involvement and hemolysis which responded successfully to treatment will be described

CASE REPORTS

Case 1

A 19-year-old woman who had immigrated to Sweden 4 years ago and had difficulties in her adaptation at work and conflict at home was admitted to the hospital 2 / hours after ingesting 60 tablets of ferrous succinate corresponding to 2 g Fe^{++} or 42 mg Fe^{++}/kg b.wt.

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On admission the patient was drowsy felt unwell and had epigastric pain. She had tachycardia (120 beats/min). BP 120/90 mmHg.

Immediately after admission vomiting was elicited One hour later she was given 1 g Desferal (desferrioxamine) i.m and then the same amount 3 hours later During the first 24 hours she was kept in the Intensive Care Unit Except for epigastric pains no sign of complication were noted.

Laboratory investigations Initial Hb 14.9 g/100 ml. Hct 40% Plasma iron 350 µg/100 ml, TIBC 310 µg/100 ml, i.e. 100% saturation (Fig. 1) WBC 6400/mm³ differential count normal. Urine sediment normal Serum electrolytes and liver tests showed normal values The variation in plasma iron concentration and the amount excreted via the urine are shown in Fig. 1 Stools had traces of blood one day after admission. One week after admission the patient left the hospital feeling completely well An X-ray of the stomach 3 weeks later was normal.

Case 2

The patient, a 26-year-old woman, mother of three children, was admitted to hospital after ingesting 150 tablets of ferrous succinate, corresponding to 11 g Fe^{++} or 180 mg Fe^{++}/kg b.wt She had had two abortions about 6 months earlier

The tablets were ingested during 3 hours prior to admission. On admission the patient was dry warm and conscious, but felt unwell BP 150/110 mmHg

Treatment with desferrioxamine 1 g i.m. was instituted 30 min after admission. When a ventricular tube was inserted, profuse vomiting was elicited The contents soon became mixed with blood. Within a few hours the patient became hypovolemic with tachycardia and falling BP and became pale and sweaty

During the first 24 hours the patient was kept in the Intensive Care Unit Partial blood exchange was performed, she was given 7 900 ml blood, and also fresh frozen plasma An attempt was made to keep the diuresis at 50 ml/hour and she was given 500 ml mannitol (15%). On the first day the patient was given desferrioxamine 1 g in 1 g and 5 g per os via the ventricular tube On the second day she received 2.5 g

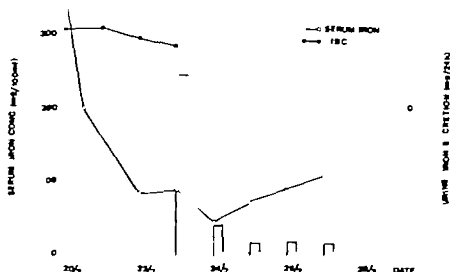


Fig 1 Serum iron, total iron binding capacity and urinary iron excretion in case 1

I. and 5 g per os in the tube and after that 1 g×4 i.m. during one week.

Laboratory investigations. Initial Hb 13.0 g/100 ml (4 h later 16.6 g/100 ml) Hct 37% (55%) Plasma iron 640 µg/100 ml (maximum 7 h later 1400 µg/100 ml). TIBC 597 µg/100 ml i.e. 100% saturation (Fig. 1) WBC 19000/mm differential count normal. Bicarbonate 4 mEq/l. Na 142 mEq/l K 3.9 mEq/l Cl 110 mEq/l Urine sediment many red corpuscles.

In the first 4 hours she felt sick and vomited fre... The total volume of fluid administered wa... h l and the fluid los 5.3 l The volume of the vomitus wa estimated to be about 6 l Initially the CVP was very low around and below zero Systolic BP 140–100–120 mmHg Temperature increased to max imum of 38 °C after 1 hours, and the patient remained subfebrile for one week. After 24 hours she was allowed to drink, and she felt well from the 6th day

After days she became icteric and polyuric (Fig. 3). She had 48 hours after admission a diuresis of 875 ml in one hour and during the 3rd 24-hour period of 8800 ml osmolarity 97 mosmol/l. The liver tests showed pathological values (Table I) culminating on the 2nd day

Hemolysis was indicated by the observation of free Hb in plasma and urine (Fig. 4) depletion of haptoglobin (Fig. 5) and slight thrombocyte depression. A slight hypokalemia was compensated. Sternal puncture was performed on the 4th and 11th days, the marrow showing no iron in the reticulum cells.

The patient was checked one month after admission. She felt well and the results of routine laboratory tests were normal.

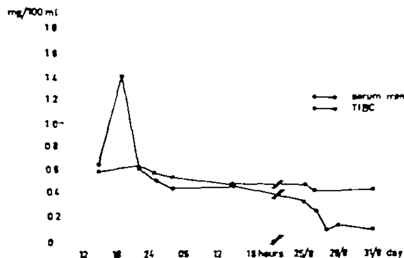


Fig 2 Serum iron and total iron binding capacity in case 2.

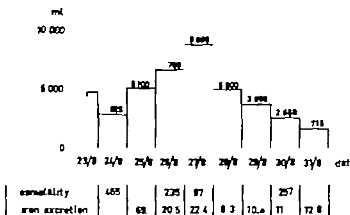


Fig 3 Urine volume (ml/24 h) osmolality (mosmol/l) and iron excretion (mg/24 h) in case ..

DISCUSSION

Iron intoxication is an unusual clinical problem in adults but it is important to know how to handle such cases because there is a high mortality rate if adequate therapy is not instituted immediately

The pathogenesis of acute iron poisoning is still controversial When ferrous compounds are given orally to different experimental animals (8 1) damage to the gastrointestinal tract has been demonstrated with small hemorrhagic spots and some edema in the stomach and more severe changes in the intestine especially the duodenum, with large hemorrhages, shrinkage of the villi and severe necrosis of the mucosa. The same findings have been encountered at autopsy in patients children and adults dying after ingestion of such iron compounds (4 6)

These changes in the intestine probably due to local irritation by the ferrous compounds may explain the absorption. It is possible that the capillaries are opened due to damage to the mucosa and that the iron can diffuse directly into the blood stream. The absorption of iron through the apoferritin mechanism is probably minimal Thus the main etiology of the iron absorption may be the capillary damage although there are some reports that thrombotizing of intestinal vessels occurs very rapidly and this mechanism would be a limiting factor (4). In our two patients there was evidence of intestinal bleeding—positive test for blood in the stools of the first patient and hematemesis in the second

Reports of damage to other organs are rare Postmortem examination of a woman who died after oral ingestion of iron (4) showed bleeding in the lungs and kidneys. Microscopic examination showed fatty degeneration of the heart, the liver and the epithelial cells of the tubules of the kidneys Our second patient showed evidence of liver damage—elevated S-GOT S-GPT and bilirubin. She also showed signs of hemolysis as judged

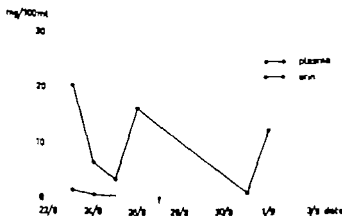


Fig 4 Free Hb in plasma and urine in case 2.

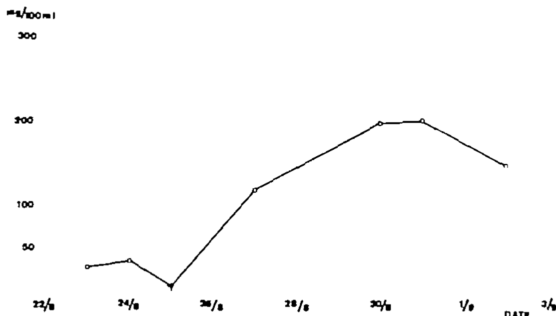


Fig 5 Serum haptoglobi concentration in case ...

from free Hb in plasma and urine haptoglobin *consumption moderate thrombocytopenia and* elevated unconjugated bilirubin. This complication has not, as far as we know been reported in the literature. The occurrence of hemolysis in this patient is difficult to interpret but hypothetically there might be two mechanisms either damage to the membranes of circulating red blood cells or an interference with different enzyme systems similar to that which can be seen after copper and lead poisoning

Hemodynamic alterations are one of the major problems in acute iron intoxication which may cause irreversible shock and death Studies on dogs (14) one hour after administration of a lethal dose of ferrous sulphate into the intestine showed a sharp decline in cardiac output a smaller reduction of arterial BP and a marked elevation of *peripheral resistance Only minimal reduction in* total blood volume was seen but the plasma volume was significantly reduced Thus early correction of plasma and blood volume is important to prevent irreversible shock and to maintain renal function to permit excretion of sufficient quantities of iron.

Our second patient showed clear evidence of hypovolemic shock, which appeared a few hours after admission She was treated with blood and plasma transfusions and attempts were made to keep her diuresis at about 50 ml/hour The explanation of the hemodynamic alterations is still obscure and several mechanisms have been suggested One is that bacterial toxins enter the blood through the damaged intestinal mucosae another

Table I *Liver function tests in case 2*

	Date						
	24/8	25/8	26/8	27/8	30/8	2/9	9/9
Total bilirubin (mg/100 ml)	13.8	17.2	13 6	5 6	2..		0.5
Bilirubin conj.	7 7	12.0	10.8	3 9	1 4		0.6
Alkaline phosphatase	5	8		8			
S-GOT	34	421	167		26	..3	17
S-GPT	31	495	489		97	68	23
LDH						236	

that unbound plasma ionic iron has a direct action on the cardiovascular system and promotes the collapse (2, 3)

Metabolic acidosis is often present together with cardiovascular collapse. The mechanism is not completely clear. It is reported that the metabolic disturbances start before the circulatory failure. It is assumed that hydrogen ion production results from conversion of ferrous to ferric ion in blood or that iron interferes with enzymes in Krebs cycle. After hemodynamic alterations with diminished cardiac output the acidosis is accentuated by tissue hypoxia and other metabolic disturbances (12)

Fever and leucocytosis are also noted in iron poisoning, probably due to tissue damage of different organs. Our second patient had fever for almost a whole week and leucocytosis (19000/mm³) and evidently she had damage of the intestinal mucosa and parenchyma cells of the liver

The most serious symptoms shock and/or coma, seem to be related to the plasma iron level. Below 500 µg/100 ml only 8% develop this picture but when the concentration exceeds 500 µg/100 ml it is more common (37%). When the concentration exceeds 1000 µg/100 ml 70% of the patients have manifest hypovolemic shock (13). It should however be emphasized that even patients with plasma iron concentration below 500 µg/100 ml can develop life-threatening shock (13 15)

The clinical features of iron intoxication can be divided into 4 stages (1). During the first 6 hours there are different gastrointestinal symptoms such as vomiting diarrhea melena, hematemesis and epigastric pain, and during this time hypovolemic shock can develop. Then there may be a period of clinical improvement (6–24 h) but the patient may still be severely ill and during the next 48 hours metabolic acidosis convulsions coma hepatic impairment and bleeding problems may arise. Several weeks later intestinal obstruction secondary to scarring can be encountered. Neither of our patients showed this late complication.

Once the diagnosis of an overdose of iron salt is suspected therapy should be started immediately. There is no time to wait for the serum iron level and furthermore the serum iron level does not always reflect the severity of the poisoning.

Vomiting should be provoked at once. Desferrioxamine treatment should be started as soon as possible (7 9 13). Desferrioxamine a potent, specific iron-chelating agent, can bind iron in the gastrointestinal tract and the complex is poorly absorbed and therefore initially 5–10 g should be given orally. Furthermore to bind the circulating iron 1–2 g every 3rd–12th hour should be administered intramuscularly to patients not in shock and intravenously at a rate not exceeding 15 mg/kg/h to patients in shock or coma. The administration should continue for up to or 3 days depending on the severity of the poisoning. In parallel with this specific therapy the patient should be monitored for shock symptoms electrolyte disturbances and acidosis. The urinary output should be followed carefully and if necessary forced diuresis may be provoked so that the kidneys can excrete sufficient amounts of iron. If the serum iron is too high exchange transfusion can be performed initially (11)

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IODINE INDUCED TOXIC DIFFUSE GOITRE

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Abstract. A report is given of iodine-induced hyper thyroidism and goitre in a patient who ingested seaweed tablets for over two years and lived in an area of iodine sufficiency. There was spontaneous remission of both hyperthyroidism and goitre three months after withdrawal of excess iodine. This case shows that neither pre-existing iodine deficiency nor goitre are necessary for development of iodine-induced hyperthyroidism and that combination of iodine-induced thyrotoxicosis and goitre can occur. Various theories for the pathogenesis of iodine-induced hyperthyroidism are discussed briefly

Hyperthyroidism is a rare complication of iodine treatment for goitre. This disease is often called Jod-Basedow although first described by Coindet in 1821 (2). Iodine-induced hyperthyroidism usually occurs in goitrous patients or in persons living in iodine-deficient areas. An increased incidence of hyperthyroidism was observed in the United States in the 1920s after introduction of iodine prophylaxis (10 15). Recently Stewart et al (20) found that iodinated bread in the iodine-poor area of Tasmania more than doubled the incidence of hyperthyroidism. Large amounts of iodine have been found to provoke hyperthyroidism in endemic areas (4) and in patients with autonomous thyroid nodules (5). Some patients with non-toxic nodular goitre living in areas of iodine sufficiency also became hyper thyroid during administration of pharmacological doses of iodine (18 21).

The present paper reports iodine-induced toxic diffuse goitre in a patient who ingested seaweed tablets rich in iodine for over two years. The patient was born and lived in an area of iodine sufficiency. There was complete spontaneous remission of both hyperthyroidism and goitre after withdrawal of excess iodine

CASE REPORT

The patient is 38-year-old female. All her four aunts have suffered from goitre, toxic in two. The patient had urinary tract infection eight years ago and has also suffered from chronic obstipation for several years. In 1969 she started to ingest seaweed tablets made in Norway in an attempt to cure her obstipation. Seaweed tablets have been on the market in this country for about ten years. In other respects her diet seems t have been well balanced. She claims that the sea eed tablets improved her bowel function and she therefore continued to take four tablets day regularly

In the autumn of 1972 nervousness and insomnia started to trouble her. She also observed increased sweating and tremor of the hands and was easily fatigued. These symptoms disappeared spontaneously after two months but recurred more intensely in Jan. 1973 after an acute respiratory infection. She still continued taking the seaweed tablets until Feb. 2, when she noticed that she had goitre. She consulted general practioner but did not mention her seaweed regimen. Diffuse enlargement of the thyroid gland and pulse rate of 90/min were found. The goitre was firm, non-tender and clearly visible. There were no eye signs. Both serum protein-bound iodine (PBI) and triiodothyronine in vitro uptake test showed highly elevated values (Table I)

She was sent to hospital for thyroidectomy. When found to suffer from hyperthyroidism she was remitted to the Fourth Department of Medicine University of Helsinki, to obtain anti-thyroid medication before the operation. This caused a one-month delay in treatment during which time her condition improved. On March 3 her hyperthyroidism was only of moderate severity and the goitre had decreased to twice the normal size as judged by palpation. Serum thyroxine determined with modification of the Murphy method (12) was elevated and so was the serum free thyroxine concentration (14). PBI had decreased to 0 90 µmol/l. No circulating thyroid antibodies were detected by the standard tanned red cell (anti-thyroglobulin) and complement fixation (anti-microsomal) techniques. There was no long-acting thyroid stimulator activity in the blood. ESR and serum

Congress Announcement

La Société Européenne de Cardiologie et la Société Française de Cardiologie organisent à Paris du 17 au 20 Mars 1975 inclus un cours en langue française sur les bases scientifiques de la physiopathologie cardiovasculaire.

Les thèmes en sont. Bases moléculaires de la contraction cardiaque La paroi artérielle normale et pathologique. Bases physiopathologiques de l'hypertension artérielle et de son traitement. Fonction sinusale et activité auriculaire Transport et utilisation de l oxygène. Ce cours est destiné à de jeunes médecins intéressés aux développements de la cardiologie fondamentale et clinique. Une connaissance précise de la langue française est nécessaire le nombre de places est limité.

Les demandes d'inscription sont reçues jusqu au 31 Octobre 1974 par Docteur René Gourgon Hôpital Lariboisière 2 rue Amboise Paré, 754 75 Paris Cedex 10

Acta med scand Vol 196 pp 41-243 1974

EDITORIAL

Medical oncology — Is it needed as a specialty*

In recent years medicine has developed along new lines and we are realizing that many malignant tumours are more wide-spread systemic than we thought a decade ago Hematology has taught us interesting lessons from the field of lymphoma, plasmocytoma and CML. In these conditions many physicians agree that eradication therapia sterilisans magna in the old sense as used by Ehrlich for Salvarsan in syphilis is no longer the immediate goal Our aim is to keep the malignant cells down. In the last decade we have become a little more optimistic regarding real healing of acute blast cell leukemia in the younger patients. It is perhaps correct to talk about eradication of the last cell when healing has lasted for many years even in the no longer treated patient.

We regard repopulation of the body organs by mobile elements from the bone marrow as normal ever since Jacobson made his first classical experiments with shielding of the spleen during otherwise lethal irradiation. Thus he saved the animals Schoenheimer wrote about the dynamic state of body constituents meaning molecules always changing in the metabolic flux We may also speak about the mobility the flux of seemingly fixed cells including microscopic tumour cells. Such a concept means that not only the knife but also cytostatic drugs antibodies, enzymes cell-seeking isotopes, etc. will have an ever increasing importance for the understanding of cancer and the war against the moving cancer cell If we believe that eradication of the last cancer cell is something for Utopia, it may be that we can still help the patient to live with his cancer One possibility might be to learn about the neutralization of the toxic substances that cause cachexia by poisoning metabolism. This may be brought about in many ways but not with knives nor with rays. Competitive action of synthetic drugs or specific antibodies directed against the presumed toxins are possibilities that must be tested systematically

Another line in modern oncology follows the leads that we may find through the study of the metabolic activities of cancer cells

Everbody nowadays agrees to the fact that the dynamic metabolic outlook on disease has been one of the cornerstones of medical progress in recent years If we were asked to give one single phrase describing what we mean by disease I would say: disturbed homeostasis It is our first duty as clinicians to restore the balance that has been disturbed and there are many ways to do this by correcting metabolic processes

Clinicians have become increasingly aware of the fact that carcinoma patients may suffer more acutely from symptoms that are described as paraneoplastic than from the direct action of the tumour The most intriguing forms of paraneoplasia are connected with what we call 1) ectopic hormone formation by cancer cells i.e the tumour produces polypeptides that are metabolically active There are other paraneoplastic phenomena that are much more obscure as far as the mechanism is concerned such as 2) neurological or autoimmune symptoms occurring in cancer patients 3) Another group of symptoms may be explained as a return to fetal metabolism 4) Finally there is the diagnostically very important group of symptoms caused by metabolic products from the normal cells that have become malignant. In this case the situation develops from normal "topic" production of active substances

Common to all these phenomena is the fact that the symptoms should be reversible when the tumour is removed This is the crucial proof of the role of the cancer cells.

What we now call paraneoplasia was first intuitively suspected by Fuller Albright, when he saw a

patient, who had severe hypercalcemia but no signs of bone metastases from a renal carcinoma. The first to collect a convincing clinical material were Plimpton and Gellhorn who described no less than 10 patients with hypercalcemia and malignancy In one patient the hypercalcemia disappeared after resection of the tumour and recurred with the metastases Bone metastases were not present and the parathyroid glands were normal at the post-mortem This severe clinical condition, that rapidly leads to death if not recognized is often easy to treat and therefore quite important from a practical point of view It has been found that it is not rare The cause must be formation by the malignant cells of *a substance identical with or similar in action to* parathormone For a very recent excellent discussion of this problem the reader might consult the June number of the American Journal of Medicine

Other examples of a similar kind are the development of polycythemia by not only renal but also by other tumours This is thought to be caused by the production of erythropoietin that is normally produced in the kidney A large number of patients who have been cured from this complication after resection of a uterine fibroma has now been published one patient also from Sweden

Cushing's disease often with strong pigmentation is said to be *more common as the result of* ectopic ACTH (+MSH) production by carcinoma than it is as a primary malady Severe hypo- t often leads to marked adynamia that may ersible Curious endocrine symptoms may se because of the ectopic production of gonadotropins etc

It seems as if all these symptoms were pretty heterogenous There are however certain clearcut features common to them all They are all caused by active substances (hormones) starting endocrine syndromes that are recognizable clinically The hormones are all polypeptides biochemically and the theory seems pretty convincing that the increased production is caused by derepression of polypeptide forming templates This would mean that there is derepression of protein forming templates (RNA DNA) *in the cancer cell*

Are there other instances of sleeping templates for protein synthesis that may be derepressed in carcinogenesis A recent finding seems most interesting. In 1944 Kai Pedersen in Uppsala discovered a new serum protein in the blood of newborn calves It was called *fetuin* and at that time it could not be demonstrated in human blood It has now been established that it occurs in the serum of patients with malignant hepatoma. *In some such sera it is* quite abundant and may even be observed directly as an α-1-fraction In many sera from hepatoma patients it is not found and it has been established that it is present in animals with hepatoma caused by some carcinogens but not in tumours casued by others Fetuin is definitely formed in the liver Its presence in hepatoma is therefore not due to ectopic production but rather to a chronological error – an awakening of a templated that became repressed at birth

Recently a patient has been observed who had bronchial carcinoma and an anemia characterized by a very marked synthesis of fetal hemoglobin (>35%) *It is known that pregnant women have a* higher hemoglobin F content than they have when non-pregnant. This points to the fact that they may respond to a factor from the fetus that derepresses their dormant template for hemoglobin F synthesis

What is important in all these conditions is I) that such paraneoplastic symptoms should lead to a thorough examination regarding carcinoma,) that even in inoperable conditions the paraneoplastic symptoms may be responsible for most of the *patient's subjective complaints. Hypercalcemia and* hypokalemia may be treated symptomatically even if the tumour cannot be eradicated and the patient *thus be restored to a much happier life for some time*

It has long been known that certain clinical symptoms may be connected with special tumours. Hypertension in phaeochromocytoma flushes in carcinoids acanthosis nigricans in tumours of the gastrointestinal tract are such examples that may lead to correct diagnosis if correctly interpreted In the first two examples metabolic invest gations have given us biochemical methods to make objective diagnoses Such examples are steadily increasing in number and importance Also for the checking of therapeutic results such parameters of metabolic type are important (cf also acid phosphatases in prostatic carcinoma)

For the recognition of all these symptoms nd of a number of others that will be discovered in rapid succession it is necessary that the oncologist ha a *good training in internal medicine especially n he*matology endocrinology and metabolism

Also treatment w th an ever-increasing number

of very active – sometimes too toxic – antineoplastic drugs must be managed by well-trained internists There is a wide-spread concern in present day society that modern drugs are too dangerous It may be important for us as doctors to remember the Latin lines from the Aeneid that describes the feelings of the Trojans regard ng the T ojan horse. They feared the Greeks even when they were bringing gifts We may well remember that "Greeks may be substituted by doctors "Timeo medicos et dona ferentes "

Well-trained specialized oncologists will diminish that fear and be able to help in conquering the most dreaded of all diseases – the malignant tumour in the widest sense of the word.

Jan G Waldenström

BOOK REVIEWS

Paraneoplastic syndromes. Annals of the New York Academy of Sciences. Volume 230 577 pp. March 1974

This symposium has resulted in volume of nearly 600 pages The attempt to cover the whole field is on the whole very satisfactory even if some interesting conditions are not mentioned, such as mastocytosis and hepatomas with cutaneous porphyria. Many topics ar treated several times in different chapters. This is partly explained by the fact that the number of active participants in this conference was very large These circumstances make it difficult to find certain subject as there is no index. The bibliography of American literature is excellent and the book is on the whole gold mine of information. With very few exceptions the speakers came from the United States European contributions to the field are often not discussed even when they are quite important.

The first part treats generalized perturbations of host physiology" with such subtitles as fever cachexia, immunology and some general metabolic disturbances. It is disappointing t find how little factual knowledge we have about these important subjects but it is also challenge to new work. The next part contains some admirable presentations regarding the problems of ectopic hormone formation. There are further excellent chapters on erythrocytosis, granulocytosis and disturbances seen in conditions with lymphocytic or plasmacellular proliferation This last subject is of course specially near to the reviewer' heart and I may well say that the presentation is admirable, both full of facts and of ideas for future research.

It is difficult to chose some special topics among the many excellent presentations of other important problems. The discussion of "our evolving understanding of the leucoencephalopathy by Richardson, who has been eminently active himself and through his group (Åström) to prove that this condition is not really direct paraneoplasia but specific viral disease.

The two chapters on renal problems one by Chisholm from Hammersmith Hospital, London and one by Fichman and Bethune Southern California School of Medicine, Los Angeles, make excellent reading and could hardly present the diverse and difficult facts in better way This is also true of Waldman chapter on protein losing enteropathies that is written by person, who is really master of his subject.

It is clear that all hapters cannot be equally good. Perhaps the most disappointing is the presentation of paraneoplastic symptoms visible on the surface e. skin and appendages. This fascinating subjects treated in such way that the reader gets confused picture of cause and effect. The author has obviously not grasped the real meaning of paraneoplasia.

The biological mechanisms behind all these clinical findings is of course the most intriguing problem in this volume. They are discussed on the last 40 pages in very stimulating way The reviewer has always regarded Gurdon experiments with enucleated and renucleated Xenopus eggs as an excellent argument regarding derepression of inactivated (sleeping ") chromosomes in the genome. Paraneoplasia as an example of specific derepression in some sleeping genes in the cancer cell of course the most probable explanation. Gurdon has proved that the genome in the nucleus from a somatic cell may be awakened and activated in the right surroundings, in the cytoplasm of an egg cell. This means the totipotentiality of each cell nucleus These findings may have been discussed.

In summary it must be said that this volume contains wealth of information and an excellent bibliography of American contributions Modern oncology has to work with these questions in the future. Even if many problems may seem to be basic the concept of paraneoplasia is clinical. It must therefore be regretted that some authors obviously have had little clinical experience In spite of this the book may be strongly recommended to all workers both in the laboratory and at the bedside who are interested in fundamental biological problems connected with cancer And who is not?

Jan G Waldenström

Pharmacology of gastrointestinal motility and secretion volume I and II Edited by Pamela Holton 665 pp £18.00 Pergamon Press Oxford, England 1973

The experimental study of gastrointestinal motility and secretion has made rapid progress during the last decade. To a large extent this development is due to advances in preparative chemistry which has made isolation of several gastrointestinal peptide hormones possible. The rapid progress in the field has recently generated several reviews dealing with various aspects of gastrointestinal motility and secretion. The present survey is an attempt to give the whole panorama of current gastrointestinal physiology and pharmacology with some additional clinical information. It is published as a section of the International Encyclopedia of Pharmacology and Therapeutics. Pamela Holton, reader of physiology at St. Mary Hospital Medical School in London has been entrusted with the editorship. She has assembled 25 wellknown scientists as authors of the 18 different chapters of the two volumes.

The book is opened with a discussion of the pharmacology of salivary secretion. Three chapters are dealing with the chemistry and biological actions of gastrin cholecystokinin and secretin. Two separate chapters are presenting different opinions on the role of histamine in the regulation of gastric acid secretion. Other parts include papers on effects of catecholamines on gastric secretion and the role of adenylcyclase and carbonic anhydrase in the gastrointestinal tract. Several other topics are also presented, such as a discussion of drugs used in the treatment of peptic ulcer and drugs for disordered motility of the alimentary tract.

The second volume is closed with a chapter on endocrine hormones producing gastrointestinal symptoms. The book does not include any general discussion of the pharmacology of the liver. The chapters mostly cover the pertinent literature up to mid-1971. Some chapters are supplemented with a short addendum covering the time until 1973. The greater part of the books is occupied by discussions of basic pharmacological and physiological mechanisms. Several of these papers are well written, and give thorough information about the present stand-point of science in respective field. However other chapters are not fully up-to-date which is partially due to the rapid pace of science making the reviewers and publishers to lag behind. The clinical topics are not given the same thorough consideration which has been paid to many of the more basic physiological phenomena. This is probably reflecting the fact that the recent advances of basic science have so far only influenced the clinical practice to a moderate degree. However there are some passages in the clinical text which are clearly out-of-date and does not reveal the significant progress which after all has been made for example in the area of diagnosis of hormone-producing tumours as a result of the introduction of specific radioimmunoassays.

On the whole the two volumes contain a lot of relevant and fresh information mainly along the field of basic physiology and pharmacology of the gastrointestinal tract. Therefore the book is of value as an encyclopedia for the experimental worker within the field. It should also be of interest for the clinician interested in new aspects of and speculation on the pathophysiology of malfunction of the digestive organs.

Lars Sjödin Stockholm

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THE VAGARIES OF ERYTHROLEUKAEMIA

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Abstract A 10-year material of erythroleukaemia from department of medicine subspecialized in haematology is presented. A total of 14 cases fulfilled the following criteria for erythroleukaemia, erythroblastic hyperplasia of the bone marrow either normo- or megaloblastic ineffective erythropoiesis, no evidence of vitamin B or folic acid deficiency relatively few myeloblasts in the bone marrow in the initial stage lethal outcome and leukaemic infiltrates in later bone marrow aspirates and often in the organs at autopsy The great variability in the clinical picture in the morphology of the red blood cell precursors and in the course of erythroleukaemia is emphasized, and the connection with other so-called myeloproliferative disorders is illustrated by the common transformation to acute myeloid leukaemia and the development of erythroleukaemia from polycythaemia vera and chronic myeloid leukaemia

The disorders commonly grouped under the designation of erythroleukaemia erythraemic myelosis or Di Guglielmo s syndrome are among the most intriguing in haematology The term myeloproliferative yndrome introduced by Dameshek (4) has been applied also to this group of diseases and it is generally agreed that one is dealing with neoplasia. The problems concerning nomenclature and classification of the Di Guglielmo syndrome have recently been reviewed (6 20)

The aim of this paper is to present a 10-year period of experience with this group of disorders from haematologically subspecialized medical department commonly treating cases of acute leukaemia. We want especially to call attention to the difficulties in establishing the diagnosis early the great variability of cases which may be categorized as erythroleukaemia, and the relationship between these diseases and other haematological conditions

MATERIAL

The patients represent cases which by routine have been registered as erythroleukaemia in this department in 196- 1971 N attempts ha been made to perform prospective study by using predetermined def nitions for entry into this series. The diagnosis has been established by the same group of haematologists as part of their routine work in haematology Retrospectively it appears that the following criteria have been essential for the diagnosis of erythroleukaemia (Tables I and II)

(1) Erythroblastic hyperplasia of the bone marrow which was either normoblastic more or les atypical megaloblastic, or just displayed abnormal cytological features. (2) Evidence of ineffective erythropoiesis from the discrepancy between bone marrow erythroid activity the degree of anaemia, and the relative lack of reticulocytes (3) No evidence of vitamin B_{12} or folic acid deficiency (4) Relatively few myeloblasts compared to erythroblasts in the bone marrow at the initial stage of the disease. (5) Short duration of the disease with lethal outcome. (6) Leukaemic infiltration in later bone marrow aspirates or in various rgans at autopsy

The mde of suspicion of leukaemia was high when the number of myeloblasts exceeded 5%. Irrespective of the myeloblast percentage, leukaemia was thought to be proven when Auer bodies were present in the myeloblasts. It is of interest to note that in some cases Auer bodies were only found upon retrospectiv bone marrow examination (see below).

The total number of patients fulfilling these criteria for erythroleukaemia in the 10-year period was 14 During the same time 112 patients were treated for acute myeloblastic leukaemia, 5 for monocytic and 49 for acute lymphoblastic leukaemia It should be noted that, with two exceptions, only patients above the age of 15 years were admitted to the Haematology Division during this period.

METHODS

All initial bone marrow samples were reexamined by one of us (S K.) The number of cells counted was 100–200. In addition, the slides were carefully screened for

Table I *Laboratory results (blood) in 14 cases of erythroleukaemia at first admission*

Case no	Age (y)	Sex	Hb (g/100 ml)	Reticulocytes (%)	MCV (μ^3)	Leukocytes/µl	Thrombocytes/µl	Myeloblast (%)	Erythroblast (%)	Serum iron (µg/100 ml)
1	17	♀	7 0	8	115	6 800	119 000	78	0	166
	75	♀	3 6	22	110	1 200	1 000	0	0	39
3	6_	♀	5 5	5	106	18 200	20 000	25	1	229
4	61	♂	9 5	8	91	500	34 000	0	8	87
5	35	♂	6 6	1		6 700	68 000	2	2	
6	41	♂	5.5	1	100	700	84 000	0	79	260
7	74	♂	5 0	3	101	400	1 000	0	0	
8	60	♀	7 5	12	1_3	600	189 000	0	0	159
9	14	♂	6 4	4	115	2 300	76 000	10	10	21
10	70	♀	6 1	6	93	600	4 400	0	2	168
11	28	♀	6.5	18	111	26 900	84 000	22	0	250
1	39	♀	8_	47	101	6 000	80 000	8	18	154
13	59	♂	3 6	4	91	400	32 000	0	0	314
14	81	♂	6 3	12	97	570	11 000	0	0	155
Normal range										90–150

Examined in another hospital just before transfer

Table II *Bone marrow results in 14 cases of erythroleukaemia (first sample after onset of symptoms)*

Case no.	Cellularity of bone marrow smear*	Myeloblast (%)	Abnormal erythroblasts* (%)	Normoblast (%)	Total erythroblasts (%)	Remark
1	++	0	51	19	70	Rather typical megaloblastic RBCP + Auer bodies present
	+	7	55	0	55	Typical megaloblastic RBCP
3	++	5	18	26 5	44.5	Moderately megaloblastic RBCP Atypical myelocytes
4	++	3	9	73	82	Atypical megaloblastic RBCP
5	+	2	1	36	37	Atypical megaloblastic RBCP quite bizarre myeloid precursors somewhat atypical
	+	1	6	60	66	Normoblast slightly atypical. Auer bodies seen in neutrophil precursors
	( )	1	1	50	51	Abnormal RBCP were megaloblastic but rare normoblastic cells appeared normal
8		2	0	64	64	No atypical RBCP seen
9		10	45	11	56	Only slight megaloblastic changes Differentiating neutrophils atypical
10	+	5	31	45	76	Abnormal RBCP more odd than megaloblastic Scattered binucleated RBCP
11	+	7	7	66	73	Only slight megaloblastic changes. Auer bodies present
1	+	3	5	89	94	Megaloblastic changes ery slight
13	+	1	0	76	76	N typical RBCP seen Very small my locytes (pseudo-Pelger cells)
14	++	9	11 5	30 5	42	Abnormal RBCP more odd than megaloblastic Several binucleated RBCP

++ = highly cellular + = normocellular (+) = hypocellular slide
Including more or less typical megaloblasts"

Serum transferrin (μg/100 ml)	Serum B_{12} (μg/l)	Serum folic acid (μg/l)	Serum bilirubin (mg/100 ml)
241	570		0 6
14	116	7	..0
266	1 128	7	1 0
	171	4 5	1 4
			1.8
17	1 090	7	0 9
	Normal	Normal*	1
305	..30	11	0 4
16			
168	360	10	0 8
390	900		0 4
	430	6	1 4
Normal*	Normal*	3	0.7
41	607	6	0 9
..30–400	150–800	4–18	<1 0

special features such as Auer bodies and other signs of dysdifferentiation in the neutrophilic line such as pseudo-Pelger cells. Chromosome studies were performed on bone marrow samples from 7 of the patients according to the method of Tjio and Whang (23).

RESULTS

Table I present age and sex distribution of the patients as well s laboratory findings at the first admission to thi department

Table II gives the result f reexamination of the first bone marrow sample The Table contai m-formation about the cell larity of bone marrow smears the numbe of myeloblast the relative frequencies of normal and abnormal erythroblasts with specification of the type of abnormal ty and special findings such as Auer bodies and dysdiffer entiation in the myeloid series The abnormal ties in the erythroblastic cells comprised both typical and atypical megaloblastic changes abnormal red blood cell precursors (RBCP) without megaloblasti changes ha e been designated as odd"

Table III show the findings in chromosome studies n se en of the patients

Fig 1 depict the course of the disease in all 14 cases tarting from the onset of symptoms and m dicating the time of the first bone marrow sample and the time when the diagnosis of leukaemia was uspected and/or established Fig shows the survival curve in the 14 cases calculated from the time of the first bone marrow sample

CASE REPORTS

Case 1

A 17-year-old woman I 196.. after ymptoms of anaemia for 3 months, she was admitted to local hospital where se re macrocytic anaemia and subnormal lues of thrombocytes and leucocytes without immature cell were discovered The bone marrow was h perplastic with atypical megaloblasts Serum B_{12} normal She was given blood transfusions and iron, but the ymptoms reappeared and months later she as admitted to this

Table III *Results of hromo om tudies I 7 ases f erythrol Laemia*

Case no	Time after admission	No of chromosomes	Site of karyotype anomaly	Other emarks
2*	2 day 6 week 11 week	42	D E & F	Ring chromosome present
3	1 day 8 week 10 week	Normal		
5	1 week	44 (80 % of metaphases)	Karyotyping not possible	Marker chromosome (80 % of metaphases) viz small metacentric chromosome half the size of group G chromosomes
6	days 74 week	Normal Normal		
11	1 eek	Normal		
12	1 week	48	E & G	All metaphases containing Ph¹-chromosomes
14	1 week	54	Karyotyping not possible	Small acentric fragment

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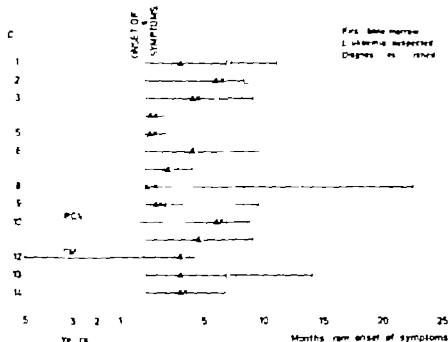


Fig 1 Course of the disease in 14 cases of erythroleukaemia from the onset of symptoms. Duration of preceding PCV and CML indicated on the left

department. There was no hepatosplenomegaly and no enlarged lymph nodes. From the blood and bone marrow findings the diagnosis of erythroleukaemia was established. After treatment with 6-mercaptopurine the myeloblasts disappeared from blood and marrow, whereas anaemia and megaloblastic changes remained. In an attempt to circumvent possible enzymatic block, treatment with thymidine (total dose 14 g/3 days) was without effect on the megaloblastic marrow. After with prednisone (40 mg/day) and transfusions was discharged but died 1 month later in febrile anaemic state at the local hospital. No autopsy.

Case

A 75-year-old woman. At the age of 50 spondylitis had been treated by several series of radiotherapy (dose unknown). In May 1965 she was admitted to the department after having suffered from increasing fatigue for 6 months. There was no enlargement of the liver, spleen or lymph nodes. The tongue was devoid of papillae and serum B_{12} moderately reduced (116 $\mu\mu g/ml$) but the Schilling test I was normal. The marrow was completely dominated by typical megaloblasts. During treatment with vitamin B_{12} some restoration of the papillae of the tongue and a marked reticulocytosis occurred but the RBC remained unchanged. The marrow was still dominated by erythroid precursors, which now were much less megaloblastic. From the finding of number of myeloblasts and a stem line with 42 chromosomes, erythroleukaemia was suspected. Therapy with folic acid had no haematological effect, and the patient received several transfusions before discharge. She was readmitted about months later with thrombocytopenia and gastrointestinal bleeding. Now the white cells in the blood and bone marrow were dominated by myeloblasts, and there was a conspicuous absence of erythropoietic cells. Death occurred in early August 1965. At autopsy the bone marrow, liver, spleen and lymph nodes were infiltrated with immature mononuclear cells.

Case 3

A 6_-year-old woman. Ten years earlier the patient radically operated upon for carcinoma of the uterus. Radiotherapy (800 R) was given 5 years before admission because of recurrent axillary abscesses. She was admitted to the department in June 1965 after having suffered from symptoms of anaemia for about 5 months. There was no hepatosplenomegaly or enlargement of the lymph nodes. Erythroleukaemia was suspected but the patient was only treated with transfusions. Three weeks later stenosis of the trachea with increasing stridor demanded tracheostomy. Tracheal biopsy showed infiltration with immature mononuclear cells. The blood and bone marrow were now dominated by myeloblasts. Despite treatment with 6-mercaptopurine the condition rapidly deteriorated and death occurred a few weeks later. At autopsy the bone marrow, liver, spleen, kidneys, tonsils and mucosa of the trachea were infiltrated by immature mononuclear cells.

Case 4

A 61-year-old man. In April 1965 he was admitted to another department because of anaemia, discovered by routine examination. From blood and bone marrow examinations erythroleukaemia as well as myelofibrosis was considered. He underwent exploratory laparotomy because stenosis of the ileum was suspected. This was not confirmed. When he was seen in this department in August 1965 there was no hepatosplenomegaly and no

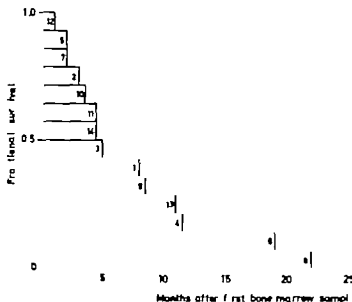


Fig. 2. Survival in 14 cases of erythroleukemia from the time of the first bone marrow sample. The numbers refer to the case reports and the hatching indicates cases treated with cytotoxic agent.

enlargement of lymph nodes. As he gradually developed pancytopenia and hypocellularity of the bone marrow treatment with prednisone (40 mg/day) was instituted. Later on, when haemolysis as documented by ^{51}Cr labelling (blood $T\frac{1}{2}$=10.5 days, splenic accumulation) splenectomy was made. Shortly after the blood showed an increasing shift to the left in the myeloid series and he died 1 month after the operation. Autopsy revealed hyperplastic bone marrow with dominance of erythroblasts, many of which showed multinuclearity. Small leukaemic infiltrates were seen in the liver, lymph nodes and adrenal glands.

Case 5

A 35-year-old man, who was admitted for pancytopenia to this department in Feb. 1966. For 2 weeks he had suffered from fever and pains in the chest. There were enlarged lymph nodes in the neck and in the axillae but the liver and spleen were not enlarged. Bone marrow examination and chromosome studies aroused suspicion of acute leukaemia. Therapy with 6-mercaptopurine was instituted but his condition rapidly deteriorated with pancytopenia. A new bone marrow sample was dominated by erythroblasts, most of which were megaloblasts. Many atypical myeloblasts were also present, confirming the diagnosis of acute leukaemia. Death occurred in April 1966. No autopsy.

Case 6

A 41-year-old man. In June 1969 he received transfusions in another hospital after having suffered from anaemia for 3 months. On admission to this department 1 month later physical examination was normal. The bone marrow material was very scanty but sufficient for the diagnosis of erythroleukaemia. Auer bodies were present in the myeloblasts. The patient was treated with vincristine and methotrexate without any effect. During the following 17 months he was readmitted 13 times for transfusions. He had no fever or haemorrhagic diathesis. The bone marrow was still hypoplastic and consequently cytotoxic treatment was not reinstituted. From August 1970 there was progressive pancytopenia and increasing number of myeloblasts in the blood but he was relatively well until Feb. 1971 when he suddenly developed a subdural haematoma. The haematoma was evacuated, but the patient died a few days later. At autopsy the bone marrow was extremely aplastic. There was moderate splenomegaly but no immature myeloid cells were seen in the organs.

Case 7

A 74-year-old man with symptoms of ischaemic heart disease for 4–5 years. For about 1 month he had suffered from anaemia when admitted to a local hospital in 1967. A severe pancytopenia was discovered and treatment with prednisone (30–60 mg/day) was instituted. For a short period he received treatment with vitamin B_{12} and folic acid. The condition remained unchanged and when bone marrow examination showed erythroleukaemia, he was transferred to this department. There was no lymphadenopathy or enlargement of liver and spleen. A new bone marrow examination confirmed the diagnosis by showing hypocellularity with predominance of bizarre megaloblastic erythropoiesis as well as a significant number of abnormal myeloblasts and promyelocytes. Treatment with methotrexate and vincristine was attempted without result and 1 month later he died from pneumonia and septicaemia. Autopsy showed lobar pneumonia and hypoplastic bone marrow. No leukaemic infiltrations were found in the organs.

Case 8

A 60-year-old woman. In 1968 she was admitted to the Department of Dermatology because of chronic eczema.

were present In addition the serum concentration of vitamin B_{12} was reduced and the patient s tongue was devoid of papillae There was some doubt concerning the diagnosis but a trial with vitamin B_{12} was made. This resulted in normalization of the epithelium of the tongue and a reticulocytosis which however was not followed by a rise in the Hb concentration. Soon the picture of the bone marrow changed to a myeloblastic pattern A ring chromosome which was present already when the patient was suspected to have pernicious anaemia remained throughout the course of the disease even at the time when the bone marrow was completely dominated by myeloblasts.

The fourth interesting case is patient 8 There was an early suspicion of erythroleukaemia but no definite diagnosis could be made The alternative diagnosis was haemolytic anaemia and prednisone was given resulting in a very definite improvement of the general condition and rise in the Hb concentration. However some months later it became apparent from blood and bone marrow cytology that she had AML. With mild antileukaemic chemotherapy and transfusions the patient was kept alive for 2 years after the first bone marrow examination. It should be noted in this context that prednisone is not or only occasionally effective in AML, of which we consider erythroleukaemia to be a variant

al features

term erythroleukaemia was first coined by Di :elmo in 1917 (10) who later extended the concept to other conditions which we now know are not neoplastic (11) Dameshek (4 5) later tried to make a summation of the various neoplastic diseases of the bone marrow such as the acute leukaemias including erythroleukaemia, CML, PCV myelofibrosis etc under the general heading myeloproliferative disorders There is no doubt that many of the neoplastic haematological disorders are intimately connected Without going into a detailed discussion of this vast problem we should like to point to the fact that several patients in this series confirm the intimate linkage between the various neoplastic haematological disorders Thus patient 10 had PCV terminating in erythroleukaemia. This course has been described previously (2, 7 2_) The transition from CML to erythroleukaemia (patient 1) has also been reported before (15 19)

The frequency and types of chromosome abnormalities found in our study are in accordance with earlier reports (3 14) In case 2 it was demonstrated that the same marker chromosome was present at the time when the bone marrow was completely dominated by erythroblasts and at the time when the marrow was predominantly myeloblastic Regarding case 1? it was noted during the erythroblastic phase that all metaphases contained two Ph chromosomes

Although it is very much used the term myeloproliferative disorder" seems less appealing to us. The point is that in many of these disorders the marrow cells are not growing faster but rather more slowly than in the normal state (13) This is also the case in erythroleukaemia (1). Therefore we would rather as a general denominator for these conditions propose the term *neoplastic disorder of the common haemopoietic stem cell*" Dependent on so far unknown circumstances the defect may preferentially lead to involvement of one of the cell lines or the picture may be that of a more generalized bone marrow disorder The limits are not sharp and during the course of the disease changes from one type to another may easily occur The diagnosis may in many instances depend on the stage of the stem cell disorder in which the patient is seen by the physician.

It has been shown that CML arises from a change in the common haemopoietic stem cell (24) and there is also good evidence that AML including erythroleukaemia arises in this cell (16 17) This may be true also for myelofibrosis (18) As regards PCV no definite evidence exists but maybe the more refined methods of chromosome studies by the banding technique will shed light on these problems.

In some respects erythroleukaemia shows differences from other acute leukaemias but perhaps this is merely an expression of how the influence of external factors upon human leukaemias can be studied in vivo Thus control mechanisms of erythroid proliferation in erythroleukaemia are not completely lost as determined from the marrow response to blood transfusions (1 8) also the pattern of marrow cell growth in itro resembles more closely that of a preleukaemic state (9)

Fig 2 shows the fate of the patients after the first bone marrow sample It is seen that the survival follows the usual survival of leukaemias i.e an exponential function It is also apparent that it

made no difference whether or not chemotherapy was given. It must be noted in this context, however, that the chemotherapy administered was deliberately not very intensive in view of the generally accepted fact that erythroleukaemias are particularly resistent to chemotherapy. In addition it must not be overlooked that the series runs from 1961 to 1971, in the early part of this period some of the now very efficient drugs were not yet available. At the present time, with a larger choice of drugs, a more active therapeutic attitude towards erythroleukaemia may be preferred.

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APLASTIC ANAEMIA. CONSIDERATIONS ON THE PATHOGENESIS

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Abstract Modern concept of haematopoiesis and its regulation are reviewed. Observations from several forms of aplastic anaemia are analysed in the light of this new knowledge. The pathogenetic mechanism in the aplasia caused by chloramphenicol is probably stem cell damage *of an autoimmune or idiosyncratic* nature. Chlorpromazine aplasia is apparently due to constitutionally defective DNA synthesis so that normal compensatory reaction cannot take place against the inhibiting effects of the drug on DNA synthesis. The chlorpromazine aplasia is normally transient, since transit cells of the bone marrow—and not the stem cells—are affected. The hepatitis-associated aplasia may possibly be precipitated by an autoimmune attack on multipotent stem cells or on transit cells of one or more cell lines after virus-induced change of antigenicity of the cell surface. The thymoma-associated aplasia may likewise be due to autoimmunity directed against transit cells. Finally there are indications that the Fanconi aplasia may be pathogenetically heterogeneous, with constitutionally defective stem cells, transit cells, or stromal cells. Otherwise stromal failure rarely seems to play a role in human aplastic anaemia. Furthermore, maturation arrest probably does not exist. Neither stem cell exhaustion nor situations with missing or defective haemopoietin apparently produce aplasia on their own. A research strategy supplementary to the routine haematological investigations, is proposed. It is recommended that modern immunological techniques in particular should be applied in combination with stem cell assays.

Aplastic anaemia is characterized by a hypocellular or acellular bone marrow and by haematopoiesis which is grossly insufficient in bone marrow as well as in extramedullary sites. This anaemia often runs a chronic course and the majority of the patients die from infection or bleeding.

The most important causes of aplastic anaemia are as follows:

Acquired 1) Physical agents (ionizing radiation) 2) Chemical agents (*a*) dose-dependent, e.g. benzene, cytoxic drugs (*b*) idiosyncratic, viz. dependent on individual susceptibility, e.g. chloramphenicol, antiepileptic drugs, chlorpromazine 3) Associated with virus hepatitis 4) Associated with thymoma 5) Idiopathic. *Constitutional*: Fanconi anaemia with variants.

Almost half the cases are idiopathic and the majority of the remaining ones are caused by drugs.

In most cases very little is known about the pathogenesis of the disease. This is to a large extent due to lack of convenient animal models. During recent years new relevant methods have been developed, however, and the possibilities for thorough study of probable pathogenetic mechanisms has improved considerably.

RELEVANT EXPERIMENTAL FINDINGS

Results obtained by experimental haematologists during the last decade may throw some light on the pathogenesis of aplastic anaemia. The attention has more and more been focused on the stem cells—their properties and growth regulation.

It is now firmly established from experiments with rodents that multipotent haematopoietic stem cells do exist (Fig. 1) (37). The distinguishing feature of stem cells is their ability to give rise both to differentiating daughter cells and to new stem cells. *The differentiating* and maturing cells will on the other hand lose—probably gradually—the capacity for self-renewal. These members of the transit populations are destined to die when their period as functional end cells is over. Some of the mature lymphocytes are apparently an exception from this rule, since they may renew themselves during an immune reaction. Furthermore, some lymphocytes are *potentially very long-lived* and this explains why they are the only remaining blood cells in significant numbers in chronic aplastic anaemia.

Normal haematopoiesis during steady state conditions can—at least in the mouse (4) and in the rat (20)—take place even though most of the stem cells do not proliferate. It is sufficient that a minority of them, perhaps 15%, are in generative cell cycle, dividing or preparing for division. The existence of a large reservoir population of non-dividing stem cells might explain why

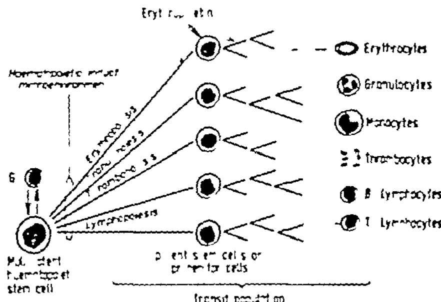


Fig. 1. Haematic cell formation from multipotent stem cell. The hierarchy of multipotent and unipotent stem or progenitor cells and the established regulating factors.

some aplastic conditions do not manifest themselves until weeks or months after the action of toxic agent, namely when dormant stem cells have been activated in order to maintain normal haematopoiesis.

There are large gaps in our knowledge about the *regulation of haematopoiesis. Erythropoietin permits* the unipotent erythroid progenitor cells to mature further to the stages of morphologically recognizable erythroblasts. It probably also increases the rate of proliferation of these progenitor cells and their more descendants (29 39 46). There is good evidence he existence of an analogous *thrombopoietin* (37) the existence of specific *granulopoietin* (37) not been conclusively proved. However it has not en possible to associate any of the clinically important aplastic anaemia with defective poietin mechanism.

Very suggestive evidence indicates that the *microenvironment* of the stem cells may influence both their direction of differentiation and their proliferation rate. For example transplanted stem cells settling in the spleen of an irradiated mouse will give rise to more erythropoiesis than granulopoiesis whereas the opposite situation is found in the bone marrow (58).

Experiments with two types of hereditary anaemia in the mouse, i.e. the W (W/W) and the Steel (Sl/Sl^d) anaemias, have given information on possible pathogenetic factors in aplastic anaemia. If normal wild-type (+) bone marrow cells are injected into lethally irradiated normal or unirradiated W anaemic mice, some of the stem cells will settle in the spleen, start proliferating and give rise to large clones of blood cells. After one week some of the clones located near the splenic capsule will be large enough to form visible node. Deeply located and smaller nodes can be examined by microscopy. This is the now classical spleen colony assay for stem cells (54). The results obtained when different combinations of + W/W and Sl/Sl^d mice were used in this assay are given in Table I. The most likely interpretations of these experiments are that in the W mice there is a genetic defect that prevents the *rapid formation of large clones of own cells*, whereas the Steel mice have normal stem cells. Here a hereditary defect in the stroma disturbs the cell growth. These interpretations have received additional support from other types of experiment (48). The importance of the stroma has also been demonstrated in experiments where spleens containing stem cells were transplanted subcutaneously, to the peritoneum, or under the renal capsule (Table II). The finding that a W spleen graft can improve the anaemia of Steel mouse indicates that blood-borne normal stem cells of the recipient can find fertile soil for haematopoiesis in the normal stroma of the graft. Several investigators have confirmed that haematopoietic progenitor cells are blood-borne in both man and mice (3 16 30 34).

These experiments with anaemic mice therefore point to two potentially important mechanisms in bone mar

Table I. *Formation of haematopoietic spleen colonies by bone marrow cells from normal or anaemic mice injected into irradiated normal or anaemic recipients*

	Recipient		
Donor	+	W/W	Sl/Sl^d
+			
W/W	Microcolonies	Macrocolonies	
Sl/Sl^d		+	−

Table II. *Effect on haematocrit of spleen trans plantation between normal and aemic or be tween different kinds of naemic mice*

Donor	Recipient	Improved (n)
f	Sl/Sl[d]	13/15
f	W/W	5/6
Sl/Sl[d]	W/W[v]	8/9
W/W	Sl/Sl[d]	8/10

Normal splenic stroma transplanted to Sl/Sl[d] mice may be colonized by circulating, normal, host stem cells, hereas normal stem cell present in the grafts given to W/W[v] mice may colonize the normal haematopoietic stroma of the hosts. Modified from Bernstein (8)

row aplasia, i.e. too few or defectiv stem cell on the one hand, and defective haematopoietic stroma on the other

M turation arrest of the bone marrow cells ha been claimed—and widely accepted—to be an important pathogenetic factor (13). There is, however no sound experimental evidence for aplastic anaemia being the *result of a deficiency of maturation factors or th* presence of maturation inhibitors (11). On the other hand tw alternativ explanations can be offered t ex plain the myelogram in such cases.

The first is that different cell types carry pecific cell surface antigens (24). Within the haematopoietic ystem there exist separate and specific surface antigens, for example on granulocytes, T lymphocytes, B lymphocytes and macrophages (44 45 53 56). The concentration of these surface antigens may increase ith increasing maturation of the cells, as show for erythrocytes in Table III. Nevertheless it is possible that stem cells, too possess at least one almost private antigen (25). The implications would then be that humoral or cell-mediated autoimmune attack could selectively eradicate mature and semimature cell of one or more cell lines. I other cases the stem cell or stroma cells might be preferentially damaged.

An alternative explanation of maturation arrest could be that *the myelogram* represents "snapshot of re-

Table III. *Antigen density on arious cell types from foetal liver as d termined by electron mic oscopy after pplicatio of ferritin-labelled anti-erythrocyte antibodie*

	Antigenic sites/μm^2
Liver epithelial cells	70
Proerythroblasts	90
Polychromatophil erythroblasts	40
Erythrocytes	500

Adapted from Minio et al. (34)

generatin haematopoietic proces that ha just eached for xample the metamy locyte stage (49)

In some types of aplastic anaemia g m benzene poisoning the aplastic stage is eached after phase of bone marrow hyperplasia This progression of he dis ease might suggest that long-lasting and stimula to pr liferation and differentiation ould *hau t th tem ell pool* with subsequent apl s result This suggestion gain support f om experiment with serial bone marrow transplantation het ex lethally irradiated yngeneic m e After 3-5 bone ma row transfers the protectiv capacity of the marrow apparentl lost (3) Although the stem cell of such transplant seem to grow poorl in bone marro the growth rat in the spleen is apparently normal F rthe If they ar let lone for some week in the bone marr of the irradiated hosts, they recover normal prol feratin capac y (57) Finally the total stem cell population of old mice not reduced (3) These findings indicat that tem ell exhaustion onl becomes important when an additional pathogenetic factor e ist such radiation damage of stromal ll

The pathogenetic mechanisms ar fairl ell elucidated in the aplastic condition caused *radiation nd hemic als* that damage haemat ie *stem cells in dose-dependent* y (14)) possible that stromal damage may be import the acute radiation syndrome nc it is know I animal experiments that rather modest radiation d s w ll lead to rupture of bone marrow sinuses and bre k ing int the parenchyma (1) F rthermor secondar aplastic phase due to fibrosi and scular damage produced in rat by large doses of X-ra irradiation experiments by Knospe and Crosby (27) The ha suggested that many cases of hitherto one plained bone marrow failure may depend on stromal damage when aplastic region persist in marrow ca ities that are otherwise normal or hyperplastic I one of their ex perimental models aplasia persi ted after supralethal irradiation and transfusion of allogeneic but not syngeneic marrow This was interpreted as indicating ynergistic effect between radiation and graft ersus host reaction against stromal components However the possibility was not excluded that stem cell proliferation w suppressed for other reasons (19 35 5) and not because the microcirculation w damaged Therefore the only xperimentally verified condition of bone mar row failure caused by stromal insufficiency is the abov mentioned high-dosage irradiation aplasia (50)

CLASSIFICATION SCHEME FOR APLASTIC ANAEMIA

A classification of possible pathogenetic factors in aplastic anaemias listed in the introduction can now be attempted. 1 Ionizing radiation. 2 Chemical cyt toxicity ( ) direct, dose-dependent, (b) conditional (idiosyncratic), ( ) immunological 3. Viral cytotoxicity (a) direct, (b) immunological. 4. Autoimmune cytotoxicity 5 Cell exhaustion 6 Hereditary defect It should be noted that any pathogenetic factor that causes chronic aplasia by attacking the transit populations of

bone marrow ells must act ontinuously A single damaging event of limited duration and great intensity may be sufficient when th attack is directed against stem cell or stroma

The classification of the linically most interesting types of aplastic anaemia into thi scheme cannot yet be carried out with an degree of confidence Clini cal observ tion gi some lues however so that some qualified guesses can be made

SEMISPECULATIVE CLASSIFICATION OF CLINICALLY IMPORTANT APLASTIC ANAEMIAS

The aplastic anaemia caused by hloramph icol will most often run a chronic and fatal course but recovery has been reported (4) In a few cases it has been cured by transfu ion of haematopoietic cells (Hjort and B yum personal communication) This indicates that the stem cells are the target cells of the pathol gical proces The few remaining granulocytic progenit rs seem capable of d iding and differentiating normally in culture (31) and the H-thymid ne labelling index of erythroblasts in itro is apparently within the normal range (40) These findings are additi nal evidence for a stem cell disea e

The aplasia may often arise even after a small total dose of chloramphenicol or late after finishing the course of drug treatment (60) A dose-dependent and revers ble p ncytopenia may also be seen er chloramphenicol, probably because the drug hibit the attachment of m RNA to membrane bound ribosomes leading to a defecti e protein synthesis in mitochondria (4 60) It is unlikely that the same mechanism works in the dangerous and irreversible form of bone marrow depression (60) since there is no e idence for abnormal accumulation of chloramphenicol in such patients The late occurrence after stopping the drug also argues against this possibility

The two most probable mechanisms of damage therefore seem to be autoimmunity and drug idiosyncrasy A model for the latter mechanism found among the haemolytic anaemias (9) It is conceivable that a hereditary defect in stem cells makes them susceptible to damage by certain chemical compounds However one would expect that an enzyme defect that predisposes to chloramphenicol damage of stem cells should also be present in the more mature cells These cells would then also be susceptible to damage but it seems that they are generally unaffected (9) It has been reported however that rather high concentrations of chloramphenicol added to bone marrow cultures from formerly aplastic patients may depress DNA synthesis to a slight extent A similar depression was found for bone marrow cells from tw fathers, but not from the mothers of aplastic patients Even higher doses were needed to affect normal control marrow (60) Since the doses were quite high (25–50 µg/ml) and since the majority of the stem cells probably do not engage in DNA synthesis during the period of drug exposure these studies do not gi e an unequi ocal answer t the question of the pathogenesis of the aplasia

Except f r the reports of two pairs of identical twin suffering from chloramphenicol aplasia there is no evidence for a genetic predisposition (9 40 43) E en if there was a genetic element this would not sol e the dilemma because the immune response to specific antigens is clearly influenced by genetic factors as shown by the existence of responder and non-responder strains among rodent

On the other hand the occurrence of a relative bone marrow lymphocytosis and plasmacytosis (40) and patchy distribution of the aplasia (60), maturation arrest (9) involvement of one cell line only (43) and during the early stages haematopoietic cell hyperplasia (9 60) is perhaps best compatible with an autoimmune mechanism. There are indications that autoimmunity is important in at least some types of aplastic anaemia, since some patients have improved after unsuccessful bone marrow transplantation. The feeling is that this might ha e been due to the immunosuppressive conditioning of these patients (5)

This autoimmune mechanism might be similar to one of those proposed for methyldopa-treated patients with haemolytic anaemia (59) Chloramphenicol or one of its metabolites could act as antigen after being bound to a soluble macromolecule or to a stem cell antigen. The humoral or cell-bound antibodies would then be directed against chloramphenicol (metabolite) or against the altered stem cell antigen, and they might in both cases cross-react with normal stem cell antigen. Alternatively chloramphenicol (metabolite) might induce immunocompetent cells to commit the "horror autotoxicus"–reacting against normal stem cells.

The autoimmune reaction that can be triggered by methyldopa (and possibly also by chlor amphenicol) may be short-lived after discontinua tion of the drug Therefore the existence of the above mentioned hypothetical mechanisms is not contradicted by the few successful bone marrow transplantations performed in chloramphenicol aplasia

The bone marrow aplasia caused by *klor promazi e* is fairly common among patients in mental hospitals (42) Blood examinations disclose agranulocytosis and in some cases thrombocytopenia. The bone marrow depression is dose-dependent. The condition is easily reversible so that anaemia rarely develops These findings strongly suggest that chlorpromazine affects the transit populations of the bone marrow After nor malization the patients can take moderate doses of the drug without serious relapse This argues against immune mechanisms and rather indicates an idiosyncratic reaction It has been shown that bone marrow from such patients has a subnormal uptake of H-thymidine in vitro even in the absence of the drug, suggesting a constitutional error in bone marrow cells On the other hand chlor promazine normally inhibits growth in several types of tissues It therefore appears that indi iduals w th constitutionally defective transit populations n the bone marrow will not be able to compensate for the growth-depressing effect of chlorpromazine (4?)

The aplastic anaemia connected with *virus hepatitis* often occurs when the hepatitis is regressing or has been cured and the attack of hepatitis has often been a mild one (1 10 21 22) Successful bone *marrow transplantation (? 5?) is evidence* against stromal damage and suggests stem cell dis ease Since only one cell line may be hypoplastic (1 22), the main attack may some patients be directed solely against transit population. It has been suggested that the bone marrow is damaged by normal doses of substances metabolized in the liver but accumulation of such substances is n-likely since the liver may not be detectably damaged when the aplasia is discovered (10, 22) Autoantibodies to thrombo- erythro- and leukocytes have been demonstrated (6) and the effect of glucocorticoid treatment has sometimes been satis factory (10) Furthermore se eral types of virus can infect haematopoietic cells. Abnormal karyotypes, which ould have been caused by virus infection ha e been found in hepatiti aplasia (1) A reasonable hypothesis is therefore that hepatitis virus infects haematopoietic stem or progenitor cells so that new antigens e.g. coded by the irus, appear in the cell membrane making these cells immunogenic

About 5% of patients with a *thymoma* have a haematological disease (18) This may be an aplastic anaemia, but more often it is an isolated erythroid hypoplasia Accordingly the pathogenetic process may be directed against a transit population The disease is prevalent in older people, and there is a connection with diseases considered to be of autoimmune nature (18 47). The occurrence of antinuclea antibodies (28 47) and a positi e direct Coombs reaction (47) fur ther support the existence of an autoimmune mechanism. Some patients improve when the tumour is removed (47) This indicates that the bone marrow condition was influenced by the thymoma. Since the tumours can belong to several histological types (18) it is probable that they cause the bone marrow aplasia indirectly by their effect on the remaining thymus tissue

The Fanconi anaemia is probably a recessiv hereditary disease (23 41) It often manifests itself about the age of 8 and a prominent feature is the great variability of both the haematological findings and the type and degree of associated malformations (23 26 41) A large number of chromosome defects can be found in PHA-stimulated lymphocyte cultures (51) and sometimes in fibroblast cultures (26 51) There appears to be n increased tendency to so-called malignant transformation by SV40 virus in fibroblast cultures from Fanconi patients (26). Bone marrow mitoses may or may not be abnormal in vi (26 51) Both megaloblastoid feat res and haemolytic anaemia ha bee re ported (26 41) It has bee suggested that a tendency to chromosomal breakage is the hereditary defect (23) A cytopathic lesion could pos sibly occur either in stem cells one or more transit cell types or in stromal cells

PROPOSAL FOR A RESEARCH STRATEGY IN CASES OF APLASTIC ANAEMIA

Hypothetical pathogenetic mechanisms for several types of aplastic anaemias ha been give above Some of the mechanisms proposed are speculative and it is therefore importa

rational strategy designed to unra el the secrets of these disorders Accordingly some procedures supplementary to routine haematological investiga tions will be proposed Multiple bone marrow biopsies could lead to the detection of patchy haematopoiesi and to a more meaningful quanti ficati n of lymphocytic and plasma cell infiltra tions A greater number of anaemias caused by hepatiti virus or by thymoma might be found if hepatitis and thymoma are pecifically sought for with X-ray examination of mediastinum, virological examination and li er function test Cytogenetic analysi may be mandatory in order to diagnose cases of Fanconi anaemia not accompanied by ther malformations (6) If possible immunological tudies should probably be done in most cases These include one or more of the foll wing procedures humoral and cell-mediated responses to haematopoietic stem and transit cells in the presence and absence of the suspected offending agents antinuclear factors antierythropoetin antibodies

Sometimes it may be worthwhile to investigate the cell kinetics of the transit populations so that e.g. so-called maturation arrest could be more accurately described Relevant tudies might include H-thymidine labelling in itro cell life span determinations nd mea uring of ⁵⁹Fe kinetic in ivo Finally it is mportant to know the quantity and proliferati e rate of haematopoietic stem cells and cell line progenitor cells There exist at pres two differ nt say procedures for such cells hich can be pplied to human material i e the diffusion hamber culture technique (7 15) and the agar lony technique (36)

New variant of aplastic anaemia will certainly appear in the future since new drugs are introduced at a r pid rate This threat and the fact *that most of the acknowledged types of aplastic* anaemia are insufficiently elucidated underlines the necessity for establishing techniques which may disclose the relevant pathogenetic mechanisms

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RED CELL .3-DIPHOSPHOGLYCERATE IN PATIENTS WITH HYPERTHYROIDISM BEFORE AND AFTER TREATMENT

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Abstrac Red blood cell .3-DPG has been found to be increased in group of 28 hyperthyroid patients. From careful analysis of the results taking into ac count Hb level the increase seems to be non-significant According to the results of the present work red cell .3-DPG annot be used parameter to evaluate the result of treatment in hyperthyroidism, advocated by previous investigators.

Over 40 years ago Bansi and Groschurth ( ) were the first to observe that the oxyhemoglobin curve of hyperthyroid patients is displaced to the right. Lately this observation was confirmed by Gahlenbeck and Bartels (8), who also obtained the same results when triiodothyronine was administered to healthy persons Gahlenbeck et al (9) found through experiments in vi o and vitro that rat blood exposed to triiodothyron ne howed de creased oxygen affinity I 1967 two groups f researchers Chanut n and Curni h (4) nd Benesch and Benesch (3), found independently of each othe that an increase of red cell organic phosphates re sults n displacement of the oxyhemoglobin dissociation curve to the right thus mproving the capability of the red cell to del ver o ygen to the tissues. .3-diphosphoglycerate (.3 DPG) being the major portion of intracellular organic phosphates has recei ed great ttention n the last few years Duhm et al (5) found normal red cell .3-DPG in rats after administr tion of triiodothyronine. Erythrocytes of healthy persons ncubated with thyroid hormone were found by Sny der et al (15) and Miller t al (1) to have ncreased 2,3-DPG levels. Riegel et al (14) made the observation in I healthy newborn inf nt that the red cell .3-DPG and plasma thyroxine level gradually increased during the first 4 day of life and that thereafter both gradually decreased

Astrup (1) found al es within normal l mits for oxygen affinity of Hb and red ell 3-DPG f 4 hyperthyroid patients M ller (13) r ported n pa tients w th hyperthyroidism who had d minished affinity of Hb for oxygen and increased red ell .3 DPG Eight hyperthyroid patient wer f und by Miller et al (1) to ha ele ted red cell 3 DPG The same authors suggested that 2,3-DPG may be used to follow cl nically rem ssions nd exacerbation n the Graves disease state Vallet et l (18) observed elevated red ell 3 DPG in hyperthyroid patient but of ignifi cantly lesser degree than pre ious authors when correct on was made for the hematocrit lev l

In the present work an attempt ha been made to follow the red cell .3-DPG level n hype thyroid patient before during nd after treatment when the patients had become clinically euthyroid. The aim ha been t find out whether th meas ement ould be used parameter evaluat ng the result of treatment The effect of moking on the 2,3-DPG level has been in estigated

MATERIAL

T enty-eight hyperthyroid patients were investigated 4 women and 4 men Their ages varied from 13 to 77 years (mean 48) A control group included 4 healthy volunteers 11 women and 13 men aged 16–45 years (mean 28)

The clinical diagnosis was confirmed by laboratory determinations (T ble I) PBI was leva ed in all pa ient and T in all but one. 131I uptake was deter mined in .. patient and as found to be elevated in 19 of them Standard bicarbonate as within normal limits in 8 patients. Hb concentration w slightly below or at the lower borderline in 7 patients.

Table I *Clink al and labx rat ry data of 28 hyperthyroid patients before treatment*

Pt. no.	Se	Age (y)	PBI (µg/100 ml)	T (%)	¹³¹I uptake	2,3-DPG (mmol/l RBC)	Hb (g/100 ml)	Standard bicarbonate (mEq/l)
1	♀	63	13 5	209	High	6 61	11.3	25.0
	♀	6_	19.8	18	High	6.54	11.2	4 0
3	♀	38	11 0	134	High	6.37	12.6	25 0
4	♀	65	11 9	151	Normal	6.18	11.5	23.0
5	♂	37	13 7	1 1	High	6.16	13.3	22 0
6	♀	79	18 0	206	High	6 16	1_2	4.5
7	♀	59	14 5	151	High	6 08	11.5	23 0
8	♀	35	14.5	229	High	6 04	1_9	19 5
9	♀	73	9 0	150	High	6.02	11.2	4 5
10	♀	72	11 5	136	High	5 98	12 8	4.5
11	♀	35	12 1	158	High	5 97	1_8	23.5
1	♀	79	9.8	133	–	5 71	13.2	1.5
13	♀	59	11	149	High	5 70	12.7	24.5
14	♀	34	1_0	190		5.59	13 0	25 0
15	♀	4	1 0	168	–	5.55	11 7	4.0
16	♀	63	11 6	145	Normal	5.38	1_7	4 5
17	♀	26	1 3	161	High	5.29	12 6	25.5
18	♀	39	8	1_3	High	5_4	13 7	22.0
19	♀	6_	11.5	143	–	5 19	15.3	25.0
20	♀	76	1_6	137	High	4 95	13 0	22.0
1	♀	18	11 5	150	–	4 91	14 1	23.0
22	♂	67	10.5	140	High	4.83	14 7	4.5
23	♀	67	16 8	197	High	4 82	11 7	23.5
4	♀	42	11 9	189	High	4 78	12.9	4.5
25	♂	13	17 0	220	–	4 77	1_7	20 0
26	♂	51	13 9	1 4	High	4 71	15 4	25.5
27	♀	33	7.5	13_	High	4.58	13.3	22 0
28	♀	77	1	101	Normal	4.39	13 1	22.5
Mea lues								
Hyperthyroid 48			1_57±2.8 (S D)	159±34 (S D)		5.51±0 6 (S D)		
Normal			4 5–7 3	80–170		4 88±0 4 (S D)	11 6–16 6	19–26

METHODS

Venou blood w draw from the antecubital fossa into 10 ml Vacuta ner tubes containing 143 USP nit sodium heparin Within 5 min 1 ml blood was placed i 4 ml chilled perchlori acid homogenized and extracted. The extracts were neutralized w th pota sium bicarbonate and say d for 2,3-DPG by the enzymatic method of Erik son and de Verdier (7) All analyses were performed in duplicat

Hb Hct acid base balan PBI T and ¹³¹I were determined by standard methods Student *t*-test was used for statistical analysi

RESULTS

Fifteen of 28 hyperthyroid patients showed elevation of 2,3-DPG above the normal range (Fig 1) The mean 2,3-DPG value for the normal group was 4.88±0 4 mmol/l RBC For the hyperthyroid group the mean value before treatment was 5.51±0 6 mmol/l RBC The difference between the two groups is highly significant ($p<0.001$)

No difference was found in the level of erythrocyte 2,3 DPG between smokers and non-smokers When the patients were divided into a female and a male group a higher but statistically non-significant level ($p>0.05$) was found in the first group.

When 7 patients with a Hb level slightly below or at the lower borderline were excluded the 2,3-DPG elevation in the hyperthyroid group was still significantly higher than in the control group ($p<0.005$) Taking into consideration that women have lower Hb levels than men, it could be expected that in our hyperthyroid group composed mostly of women the 2,3-DPG level would be higher than in the normal group with almost equal proportions of men and women. Therefore a new analysis was performed of the results in the female hyperthyroid

Table II *Red cell 2,3 DPG PBI and T of 19 hyperth roid patient before nd afte treatment*

PBI alues (within parentheses) for patient treated with ¹³¹I were excluded when mean values were calculat d

Pat. no.	_3-DPG (mmol/l RBC) Before	After	PBI (µg/100 ml) Before	After	T (%) Before	After	Therapy
4	6 18	5 90	10.5	5 0	160	104	Carbimazole
5	6.16	5 02	18 0	13	106	80	Propylthiourac l
9	6.02	5 76	9 0	(1 8)	150	78	I
10	5 98	6 84	11.5	(10._)	136	1_5	I
11	5 97	5 38	1_1	4 6	158	78	Carbimazole
1	5 71	6 49	9 8	5	133	77	Op +levoth ro ine
14	5 59	6.54	1 0	3 0	190	80	Op
15	5.55	4 98	1 .0	4 9	168	98	Carbimaz le
16	5.38	4 96	11 6	10 0	145	119	Carbimazole
17	5.29	5 64	1_3	5 3	161	117	Propylthiourac l
18	5_4	5 19	8	(3 1)	1_3	85	¹³¹I
19	5 19	5 1	11 5	8 5	143	83	Carbimazole
1	4 91	4 98	11.5	7 5	150	114	Carbimazole levothyro ine
22	4.83	4 85	10.5	(6.5)	140	79	I
_3	4 87	4.35	16.8	(15.0)	197	104	I
4	4 78	5 43	11 9	(4 5)	189	88	I
26	4 71	4 47	13 9	1_9	174	78	Propylthiouracil levoth r ine
27	4 98	5.38	7 5	7 5	137	130	Carbimazole levothyro ine
28	4.39	4 70	1	(5 8)	101	73	¹³¹I
Mean±S.D	5 33±0 6	5 35±0 7	11 73± 5	6 08±3 3	153±28	94±19	

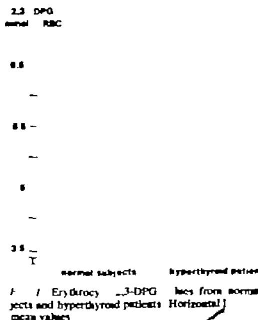
group e lud ng the patient with Hb levels at the lowe borderline or below The mean alues were 5 4 ±0 6 for 17 hyperthyroid females and 5 00±0 4 f r 11 normal females with Hb levels well w thin n rmal limit The difference is non-significant ($p>0.05$)

The mean value for red cell 2,3-DPG before and fter treatment showed no significant difference whereas the corresponding values for PBI and T showed highly significant difference ($p<0.001$) (T ble II).

DISCUSSION

The red cell 3-DPG in hyperthyroid patient was significantly higher than in the controls The magnitude of this ncrease was far from being of the same order as found by Miller t al (1) who found elev tions above th normal range n all patients nvestigated I the present study only 15 of 28 patient had alues above the normal range

Red cell 2,3-DPG is elevated in hypoxic conditions (10 11 17 19) and this ha been nterpreted as a compensatory manifestation since the oxygen transport to the tissues is mproved through displacement of the oxyhemoglobin curve to the right. Eaton and Brewer (6) as well as Hjelm (10) have reported an n erse correlation between 2,3-DPG nd Hb concentration healthy persons In the



F 1 Erythrocy 2,3-DPG lues from normal jects and hyperthyroid patients Horizontal l mean values

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ARRHYTHMIAS IN THE EARLIEST PHASE OF ACUTE MYOCARDIAL INFARCTION

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Abstract During 4-year period acute myocardial infarctions (AMI) occurred 26 times while the patients *were monitored on ECG in ou Coronary Care Unit* (CCU). The arrhythmias observed during the earliest phase were as follows: No or non-serious arrhythmias were seen in 11 cases. I 8 of them the AMI were considered to be small One patient with small AMI had ventricular extrasystoles (VES) Bradyarrhythmia was *seen in 7 cases. In 6, including all the 5 patients with* inferior or posterior AMIs it occurred during the first minutes In one with VES bradyarrhythmia developed 2 hours after the AMI. In 4 cases VES and entricular tachycardia developed later in the course. I 2 no other arrhythmias were seen Primary ventricular fibrillation was seen in 3 patients, in one as the first symptom in the others 12 and 30 min after onset. They were all defibrillated and left the hospital in good condition. Fou patients developed cardiogenic shock shortly after the onset. Two died within 40 min, the others within the next few hours. Except for the agonal rhythm disturbances no arrhythmias were seen Altogether 6 patients died and 3 more would probably have died if they had not been in the CCU when they developed the AMI. Five or more than half of these 9 patients, died or would have died withi 40 min, during the usual prehospital period. This is in accordance with other *studies and it poses probably one of our greatest medi*cal problems in terms of loss of potentially salvageable life.

More than half of the deaths from coronary heart disease (CHD) occur *within 1 hour of onset of* symptoms (? 6, 7 12). It is supposed that at least half of these sudden deaths are due to arrhythmias (10) Due to the delay between onset of symptoms and admission to the Coronary Care Unit (CCU) the clinical events occurring during the earliest phase of acute myocardial infarction (AMI) in man are largely unknown. The ideal situation for studying the earliest period is when the patients are already monitored on ECG when they develop the AMIs (3 9) During a 4 year period this happened 26 times among 25 patients in our CCU In the same period altogether 688 patients with AMIs and 243 patients with intermediate coronary syndrome were treated in the same unit.

METHODS

The patients reported were treated in the CCU at Diakonissehusets Hospital from Jan. 1970 to Jan. 1974 Three patients were still in the CCU 5 days following an AMI when they sustained new one The others had all had one or more non-severe but typical attack of chest pain without accompanying enzyme abnormalities, and with no or only minor ECG changes. One patient had been in the CCU for only 10 min the others 2–40 hours when they developed an AMI All patients n the CCU were monitored on ECG A 4-channel oscilloscope was continuously observed and all arrhythmias were recorded The diagnosis of AMI was based on sudden *onset of typical chest pain (17) very probable or pos*sible ECG changes (17) and SGOT >50 U I the earliest phase all patients were treated with morphine, oxygen and warfarin The antiarrhythmic treatment given is mentioned in the case reports.

CASE REPORTS

The patients are numbered according to their admission date and divided into groups according to the size of the AMI and to the rhythm disturbances When nothing be is mentioned they had had no known earlier AMI, *had not used digitalis or diuretics and they left the hos*pital in good condition. Ventricular extrasystoles (VES) less than 5/min are called few

Five male patients (nos. 8, 11 12, 18 and 19), 51–77 years old and 4 female (nos. 10 21 23 and 26) 58–75 years old had signs of small AMIs. Serial ECGs showed *transient ST T changes* and the SGOT values increased from 12–20 U before the AMIs to 53–110 U after Case 10 had supraventricular tachycardia about 6 min after onset with heart rate (HR) of 160/min. It lasted for only 5 min. Case 23 had frequent supra-VES with aberrant conduction 8–10 min after the onset and of 4–6 min duration. Case 26 developed VES 3 min after onset

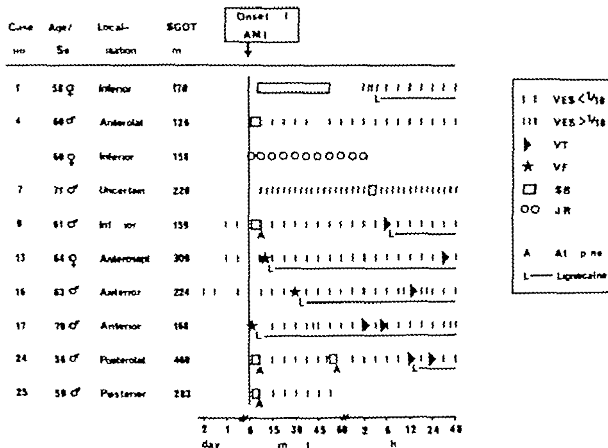


Fig 1 Arrhythmias during the first 48 hours in patient with AMI *VES* = ventricular xtrasystoles, *VT* = ventricular tachycardia *VF* = ventricula fibrillation, *SB* = sinus bradycardia, *JR* = junctional rhythm

was treated with lignocaine for few hours In the ther patients no arrhythmias were seen xcept for a few VES and no antiarrhythmic drugs were given

Th ee male patient (nos 14 15 and 20) 61–7? years old had anterior AMI with maximal SGOT values of 126–264 U Two of them (nos. 14 and 20) had had two earlier AMI and they both used digitali All 3 had only a few VES and no antiarrhythmic treatment was given Case 14 developed LBBB on the third day Seven patients developed bradyarrhythmias (Fig 1)

Case 1 developed sinu bradycardia (SB) of short duration HR 48/min about 5 min after the onset The SB may have been caused by morphine given 1 min earlier No atropine was given but late lignocaine be cause of VES

Case 4 developed SB HR 44/min 3 m after onset It lasted only about 5 min Atropine was not given

Case 5 developed junctional rhythm (JR) HR 55/min 2–3 min after onset. It lasted for about 2 hours No other complications were seen until the sustained new AMI on the 5th day while she was still being monitored on ECG (see case 6)

Case 7 developed his third AMI in our CCU He was in heart failure and had multifocal VES 1–2 min after onset. He was treated with digoxin and furosemide but not with lignocaine Two hours later he had short episode of SB HR 46/min H died 3 weeks later of heart failure

Case 9 The SB which occurred 2–3 min after onset, was abolished with single dose of atropine He also had a few VES and a short run of ventricular tachycardia (VT) which wa treated w th lignocaine

Case 24 developed SB about 2 min after onset, HR 33/min. Atropine was immediately effective but he needed a new injection 30 min later He also developed VES and VT his clinical ondition det riorated and he died on the th d day of cardiogenic shock

Case 25 developed SB about 3 min afte onset HR 35/min Atropine wa immediately effective

Three patients developed ventricular fibrillation (VF) (Fig. 1) They were all defibrillated at once with 400 Ws. All 3 also had VT later in pite of lignocaine the apy Their course wa otherwise uncomplicated

Case 13 had few multifocal VES before as w ll as after an AMI VF occurred unexpectedly 1 min after onset

Case 16 had had two earlie AMIs He had few VES before as well as after an AMI VF occurred unexpectedly 30 min after onset

Case 17 had no pai and no arrhythmias when he

suddenly dev loped VF When he woke shortly afterwards he had severe typical chest pain. Serial ECG showed unequivocal AMI.

Four patients (no _, female 75 y., no 3 male 64 y., no 6, female 60 y and no 22 male 73 y) all had very serious clinical condition with hypotension and heart failure shortly after onset. They all developed cardiogenic shock and died within 30 and 40 min, 5 and 6 hours respectively Except for the agonal rhythm disturbances no arrhythmias were seen

The events occurring at different intervals after the onset of the AMIs are summed up below

(1) During the *first 30 min* Four patients, of whom one died, developed cardiogenic shock Three patients had VF all were defibrillated T patients of whom one was in heart failure had multiple VES Six patient developed bradycardias 5 SB and one JR I one k ceased spontaneously m 3 after atropine injection, while in h continued without complications. Among the remaining 11 patients one had short attack of supra entricular tachycardia the others only few VES or no arrhythmias at all

(2) During the *next 30 min* Another patient in cardiogenic shock died and one had new short attack of SB

(3) During the *following 47 hours* (1–48 hours after onset) The remaining patients I cardiogenic shock died All the 3 patients with prior VF had VT although they w re on lignocaine therapy Among 3 of the patients with prior SB, had VT and one multiple VES Sixteen patients including all the 11 w thout prior serious arrhythmias, had only few VES or no arrhythmias at all

(4) During the period from the 3rd day until the patients left the hospital One patient with prior SB and VT died m cardiogenic shock. One patient with intractable heart failure died One patient had re-infarction Among the other patients one developed LBBB the 3 patients with prior VF had VES whereas none of the other patients had serious arrhythmias.

DISCUSSION

Only a few patients sustain their AMIs while they are being monitored on ECG in a CCU These patients have usually been admitted to the CCU because an AMI could not be excluded until serial ECG and laboratory tests were available Meanwhile they develop an AMI A few patient may also ha e a re-infarction while they are still being mon tored because of the first one In our CCU less than 4% of the patients w th AMI had it while they were being monitored on ECG

The earliest event during the AMI probably depends upon the size of the AMI (5 15) In the present material of 26 AMIs 9 were considered to be small This is higher incidence of small AMI than in our total AMI material (15) and may plain the high incidence of patients w thout serious arrhythmias In 4 patients however the clinical courses were very serious It is remarkable that none of these patients had arrhythmias

Primary VF is a ery early complication in AMI and is seldom preceded by warning arrhythmias (1 8) Three of our patients were probably saved because they w re the CCU when they developed VF In one of the patients VF was the first symptom of AMI in the other two it occurred w thout conv ncing warning arrhythmias I and 30 min after onset.

Bradyarrhythmias are also seen w th a higher incidence the earlier the patients are observed (1) especially in patients with inferior or posterior AMIs. In all our 5 patients with this localization and n one patient with anterior AMI the brady arrhythmias occurred during the first minutes in only one patient more than one hour after onset

The numbe of cases n the present study is too small to draw any certain conclusions. The true variation of the earliest clinical course in AMI may be another Our e periences are howe er in accordance w th other studies where more than half of the CHD deaths occurred during the first hour (1 6 7 1) and where a high percentage of these deaths were due to primary VF (1) If our 25 patients with 26 AMIs had been admitted to the CCU one hour after the attack, 5 patients would probably have been dead before they reached the hospital Two of these patients died from cardiogenic shock, the other 3 would ha e died from primary VF Four patients died later in the hospital Altogether 9 patient would ha e died of whom 5 within one hour

The prehospital phase of AMI poses probably the greatest single medical problem in terms of los of potentially salvageable lif (4) E en when mobile CCUs are available only 6% of the patients with AMI are seen within 30 min after onset (1) Among patients dy ng outside the hospital 78% died before ambulance was called (13) The potential profit of pecial precoronary care station i order to observe patient who display larming symptoms would also be limited by the fact tha only two-thirds of patient w th AMI ha prodr mata (14) and that among patients dying outside hospital only one-third thought these prodromata were serious nough to seek medical con ultation (15)

Establishment of both mobile CCU and pre-

coronary units together with better education of physicians and patients especially in the highest risk groups is probably the best answer to this problem today

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PRE EXCITATION IN YOUNG MEN

Incidence ome Anth opometric Data and Phy ical Work Capacity

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Abstract Sixteen young men with pre-excitation found at an enlistment medical examination of conscript (gi ing an incidenc of 1/1000) have been compared with 11 randomly selected healthy men of the same age None in the pre-excitation group had any signs of organic heart disease or history of tachycardial bout Those with pre-excitation were significantly shorter and had a slightly higher BP than the controls, but did not differ regarding physical work capacity (W_{170}) or heart volume (HV). No difference was found in these respects between types A and B pre-excitation W_{170} was, when adjusted for differences in HV significantly higher in 8 subjects, whose ECG were totally or partially normalized during exercise than in 8 subjects whom no normalization occurred

particularly as the correct timing between trial nd entricular systole is n part impaired so that the sometric contraction of one of the entricles starts before the atrial mechanical systole has ended This might give a decrease in the stroke olume at least at higher heart rates

The aim of our study has been to determine the incidence of pre-excitation in a general population and the frequency of concomitant bouts of tachy cardia and other disorders and to evaluate the consequence of pre-excitation to physical work capacity

It is nowadays generally agreed that there is an anomalous conduction bypassing the atrioventric ular node patients with pre-excitation It is often ssociated with bouts of tachycardia (4 8 10 16 20 22, 26) Pre-excitation is reported to be more common n congenital heart disease such as cardiomyopathy subvalvular aortic stenosis and Ebstei s anomaly of the right tricle (5 7 8 1.. 13 20 25 26) and some congenital cerebral disorders (4 5 1 20 1) Of subjects with pre excitation found in a hospital population 55–80% are reported to have complicating d sease (4 20) The functional effect of pre-excitation has been tudied by right heart catheterization nd other methods and a impairment of ejection in one or both entricles has been claimed (1 3 13 15 17 18 23 26) In iew of the connection with different disorders of the heart a diminished capacity for physical exercise may be expected,

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MATERIAL AND METHODS

Altogether 16 men th pre-excitation were observed in material of 16 000 conscripts, about 18 years of age consistently xamined during the course of 18 months at the Enlistment Centre Solna From the same material 112 healthy conscript with ordinary ECG ere randomly selected and used as control material A criterion of pre-excitation a δ-wave in at least tw leads and with duration of 3 csec in at least one of them was used (17). The P–Q interval turned out to be less than 12 csec in all cases. The ECG was classified as type A when the R a was the sole or the largest deflection in the right precordial leads particularly CR and CR_4 If the sole or largest deflection in the right precordial leads was an S or QS wave the ECG was classified as type B

I both groups heart olume (HV) the supine and upright positions was estimated (11) An orthostatic test was done including heart rat (HR), BP nd ECG after standing for 8 min. The exercise test wa performed the sitting position on an electrodynamically braked cycle ergometer (Siemens-Elema, EM 370-1) with stepse increasing load every 6th min up to HR of about 170 beats/min 4) The load giving HR of 170 beats/min (W_{170}) was used as measure of physical work capacity The ECG was recorded with an ink

Table I *Some anthropometric and other data in 112 controls and 16 subjects with pre-excitation*

	Control		Pre-excitation		
	Mean	S D	Mean	S D	$p<$
Height (cm)	179 9	6.5	176 3	6.4	0 05
Weight (kg)	68 3	10.0	67 7	7 8	(0.90)
HR at rest (beats/min)	66	11	69	11	(0 40)
HR in orthostatic test (beats/min)	85	13	85	1	( )
Blood volume (l)	5 6	0 7	5 1	0.7	0 05
Systolic BP recumbent (mmHg)	125.3	11.5	130 9	13 0	(0 10)
Diastolic BP recumbent (mmHg)	73 9	8.0	78 8	6.2	0 05
Systolic BP standing (mmHg)	125 1	10 1	130.3	15 1	(0 10)
Diastolic BP standing (mmHg)	81 1	7 9	81 9	7 9	(–)
W_{170} (kpm/min)	1 033	19	1 021	234	(0.90)
HV (ml)	771	118	779	108	(0 80)

jet electrocardiograph (Mingograph 81 Siemens-Elema). In repose CR leads were used and during exercise chest-head leads (9). BP was measured with a calibrated cuff on the right upper arm by auscultation. The observed value was rounded off to the nearest level of 5 mmHg. The significance of differences between groups was assessed using the *t*-distribution.

RESULTS

Sixteen cases of pre-excitation were found 5 of type A and 11 of type B giving an incidence of 1/1000 in the population consisting of 18-year-old men They had no knowledge of their abnormality None of them had any history of tachycardial bouts and physical examination revealed no sign of cardiac disease besides the pre-excitation In the pre-excitation group the mean height was 3 6 cm less than in the control group ($p<0\ 05$) (Table I) The blood volume was on an average 0 5 l smaller (mean 5 1 l S D 0 7) in the pre-excitation than in the control group ($p<0\ 05$) The resting diastolic BP in the recumbent position in the pre-excitation group was 78 8 mmHg (S D 6 2) which was 4 9 mmHg higher than in the control group ($p<0\ 05$) Systolic BP was numerically higher in the pre-excitation group than in the control 5 6 mmHg ($p<0\ 10$) in recumbent and 5 2 mmHg ($p<0\ 10$) in standing position

The mean values for W_{170} and HV in the pre-excitation group did not differ from those in the control group (Table I) During the orthostatic test there was no change of the degree of pre-excitation and no difference in orthostatic reaction regarding BP and HR as compared to the controls (Table I) There was no difference between pre-excitation type A and type B regarding W_{170} and HV (Table II)

During exercise three subjects with type A and five with type B pre-excitation had a partial or total normalization of their ECG Those with a regress of the pre-excitation signs had a broader δ wave at rest than those with unchanged degree of pre-excitation during exercise (4 5 and 4.3 csec respectively) but the difference was not significant There was a tendency to a higher physical work capacity for cases with regress in pre-excitation during exercise than for cases without but the difference (168 kpm/min) was not significant (Table III) No significant difference in HV was found The tendency to higher work capacity

Table II *Physical work capacity and heart volume in types A and B pre-excitation*

	N	x̄	S.D	p
W_{170} (kpm/min)				
A	5	1 080	144	<0.7
B	11	995	268	
HV (ml)				
A	5	746	43	<0 5
B	11	794	126	

Table III *Physical work capacity and heart volume in cases with and without regress of pre-excitation during exercise*

	N	x̄	S D	p
W_{170} (kpm/min)				
Regress	8	1 106	195	<0 70
No regress	8	938	352	
HV (ml)				
Regress	8	768	105	<0 70
No regress	8	791	116	

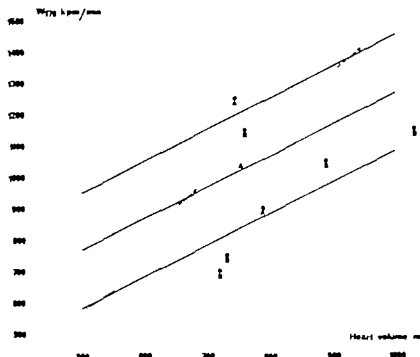


Fig. 1. W_{170} in relation to HV in 11 normals. Regression line ±S.E.M. (W_{170}= 0.98×HV+779.3. S.E.M. = 186.7. r=0.53. p<0.001). ---- =regression for subjects with regress in pre-excitation during exercise (O). —— = regression for subject with no regress in pre-excitation (●). A and B types of pre-excitation.

at a HR of 170 beats/min for cases with regress in pre-excitation during exercise was found to be significant ($p<0.05$) when differences in HV had been accounted for by means of analysis of covariance, i.e. those with a regress of pre-excitation had a higher physical work capacity at a HR of 170 beats/min in relation to HV (Fig. 1). The difference between adjusted means, i.e. the critical distance between parallel regression line, was 203 kpm/min.

DISCUSSION

The incidence of pre-excitation in the population studied by us, 1/1000, is rather low in relation to other reports of incidence ranging from 1.6 to 3.1/1000 in similar non-hospital populations mainly consisting of fairly young men (6, 8, 22). None in our material had any history of tachycardial bouts, which agrees with earlier reports that the frequency of tachycardias in pre-excitation 0–4% is low in general health surveys (16, 22). However, frequencies as high as 1 % have been reported ( ) and in hospital ECG surveys the figure is considered to be 50–70% (4, 10, 16, 20, 26). The frequency of organic heart disease in pre-excitation in this study was low (zero), which is in agreement with reports from other non-hospital populations (2, 14, 16, 22).

The physical work capacity was normal in the present material of pre-excitation, which seems to indicate that pre-excitation per se is fairly harmless. A study of physical work capacity in pre-excitation has earlier been made only by Sandberg (19), who found a moderate reduction. The difference can be explained by the fact that his pre-excitation cases were older and more heterogenous and belonged to a hospital population aware of their anomaly. Furthermore his control group was younger than the patient group. It is, however, probable that pre-excitation may have mechanical consequences at higher HR during exercise, since it was found in this study that there is a significant difference in physical work capacity between those in whom the pre-excitation diminished or disappeared during exercise and those in whom it remained unchanged or became worse.

We have no explanation to offer at present regarding the difference in body height and BP in comparison to the control group.

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HEART FAILURE AS COMPLICATION OF LITHIUM TREATMENT

P eliminary R port of a Fat l Case

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Lithium has been used in the treatment of psychiatric disorders since 1949 (ω) but as early as in 1907 there was warning I The Practitioner (11) against de pression and dilatation of the heart Few cardiac side effects have since been reported Temporary ECG changes w th flattening of the T w ves have been de scribed (3 6) as have frequent entricular extrasystoles in association w th therapeutic concentrations (10). One fatal case of interstitial myocarditis has been described by Tseng in 1971 (12). The lithium determinations in the latter case have been criticized by Schou (5). We re port fatality probably due to prolonged lithium treat ment

CASE REPORT

A 46-year-old woman with long history of episodic depressions. She had suffered from sl ght asthma for several years and, because of this had taken potassium iodine 0.3 g/day since 1968 Lithium treatment was started in April 1971 Monthly serum assays showed Li/ levels of 0.7 1 1 mEq/L She was simultaneously treated with amitryptiline 75 mg/day In March 1973 she was admitted to hospital because of weight gain of 25 kg over the past t years to 89 kg. She complained of swelling, obstipation, chillness and inactivity Lithium had been withdraw one week prior to admission

Physical findings included obesity periorbital oedema and hoarseness. The thyroid gland was not enlarged Regular tachycardia with faint sounds and no murmurs BP 120/80 Laboratory findings: ESR 8 mm Hb 12 7 g/100 ml cholesterol 360 mg/100 ml TG 115 mg/100 ml PBI contaminated T 3 70% (normal 80–170) BMR −15% TSH 58 μU/ml (normal <10) [131]I uptake in 24 h as 3% Serum electrophoresis normal No antibodies against TBG or thyroidal cytoplasma

Substitution therapy w started th thyroxine 0 1 mg/day One week later addition of triiodothyronine 60 μg/day for 14 days After 1 month therapy with thyroxine p to 0 25 mg/day ESR was 3 mm cholesterol 95 mg/100 ml BMR 22% T 3 99% and TSH <3 μU/ml. Weight loss of 8 kg. Serology against Cox sackie virus B negative.

On admission X-ray of the heart revealed marked general enlargement to 880 ml/m² BSA and light right-sided pleural ffusion ECG showed sinus tachy cardia 100/min, low oltage in the extremity leads and generally flat T w ves. N arrhythmia during the whole treatment period After 5 week of thyroxine substitution the heart size was unchanged and ther was more fluid the right pleural cav ty Echocardiography did not disclose any pericardial effusion ECG now showed higher amplitudes but was otherwise nchanged Thoracocenteses on the right side yielded 780 ml fluid

Digitalis was not given, as the heart failure was attributed to hypothyreoidism and the patient howed no linical sign of decompensation. However in spite of adequate hormone substitution cardiac decompensation dev loped and after 5 eeks of thyroxine treatment the patient began to complain of discomfort, especially of dyspnoea Because of increasing cardiac insufficiency the patient as transferred after 7 eek to the Depart ment of Endocrinology and Cardiology of Sahlgren Hospital, Gothenburg An idiopathic ardiomyopathy was diagnosed The patient as treated ith digitalis and diuretics and her ymptoms regressed A thrombosis in the right subcla ian ein as treated th heparin and dicoumarol The patient was discharged after 3½ month of hormone treatment, on maintenance with digoxin 0.25 mg 1 furosemide 40 mg 2 spironolactone 25 mg 4 thyroxine 0 15 mg 1 and dicoumarol. She died suddenly 3 weeks later

Autopsy find gs

Autopsy was performed 40 hours after death The patient was 157 cm tall and eighed 66 kg

The *heart* eighed 500 g and showed slight hyper trophy of both the left and ght chambers The myocardium was somewhat streaked and gr yish-red In the coronary arteries there er only few scattered non-calcified yellow deposit in the intima the lumina were not narrowed

Microscopic xaminat on evealed small scars in number of region in the myocardium these consisted of fairly acellular connective tissue. There were also partially anastomosing regions of oedematous connec tive ti ue containing sparse, or in places denser collec tion of inflammatory cells, especially mononuclear cell but also some neutrophilic granulocytes and an occa-

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TRANSVENOUS CARDIAC PACEMAKER AS A FOCUS OF SALMONELLA INFECTION IN A PATIENT WITH HEART BLOCK

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Abstract. A case of S typhimurium septicemia in association with permanently implanted transvenous pacemaker is described The Salmonella infection had probably been contracted long before and was possibly the cause of failure in heart conduction. Antibiotic treatment alone was without result, but after removal of the pacemaker system it resulted in eradication of the Salmonella infection and was also followed by elimination of the heart block. Three years after the operation ther is still no need for artificial pacing

Foreign material intracardially implanted is a factor predisposing for severe infections Thus septicemia and endocarditis ha e been reported in patients with prosthetic cardiac valves (11) and with intracardiac catheters for the alleviation of hydrocephalus (18) There are also some reports of septicemia (8 9) and endocarditis (5 6 17) in patients with permanent transvenous pacemakers which since 1958 are commonly used for artificial pacing (7) The bacteria most often involved have been Gram-positive cocci (5)

This report deals with a Salmonella infection in a transvenous cardiac pacemaker system, resulting in septicemia. Combined conservative and surgical treatment resulted not only in elimination of the Salmonella infection but was also followed by disappearence of a 2nd–3rd degree heart block, which had been the reason for pacemaker implantation.

CASE REPORT

Clinical data

A 56-year-old man who had experienced an attack of high fever of unknown origin in 1935 when serving in S edish naval station. At the same time several of his fellows had similar febrile illness, with fatal outcome in two. Since then the patient has had about twice year episodes of fever up to or above 40°C typically subsiding within 3 day and without any concomitant symptoms. While the history is vague concerning the 40° the attacks have been better recorded during the last two decades.

He had made some trips to Mediterranean countries and the Canaries during the last 10 years, but on these occasions he had no symptoms of gastroenteritis compatible with salmonellosis. In 1953 he suffered from repeated stomach ulcers, which led to partial gastric resection ECG has been normal and no signs or symptoms of cardiovascular diseases or hypertension ha e been noted.

On May 1 and 2, 1968 repeated Adams Stokes episodes occurred and the ECG indicated an atrioventric ular block changing between 2nd and 3rd degree A transvenous cardiac electrode was inserted and attached to an xternal pacemaker Regular sinus rhythm was established and the patient was discharged from the Thoracic Clinic one week later in good state of health There were no levations of serum transaminases and heart volume was normal according to X-ray

In August 1968 subcutaneous implantation of the pacemaker into the left abdominal wall was performed Because of purulent skin erosion in the right parasternal area, caused by too superficial position of the electrode dissection of it to more profound layer had to be made Though no pathogenic bacteria could be cultivated from the erosion, the surgical intervention was combined with ampicillin treatment Some irritation of the skin persisted during the following months and there was also tenderness over the pacemaker pocket. No bacterial cultures were performed In Dec 1969 he had chills and dizziness of the same type as before but no signs of skin infection

In Feb 1970 the pacemaker bo had been dislodged upwards in the left abdominal wall and was replaced by new one implanted in lower region of the left abdominal wall There were no signs of infection and cultures from the pacemaker pocket yielded no growth From the autumn 1970 there were continuously erosions along the electrode and nfection with S. aureus and E coli was found. On Oct. 27 1970 for

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the first time there w s growth from the lesions of Salmonella typhimurium phage type 1. Stool cultures were negative for Salmonella but the Widal test was positiv against S. typhimurium O and H antigens.

He was again admitted to the Thoracic Clinic at the beginning of Dec. There was obviously a persistent infection along the whole subcutaneous part of the electrode from the right supracla icular fossa to the pacemaker box just above the left groin. Two erosions with purulent secretion again yielded growth of S. typhimurium. A ex sion of the erosions was performed and, in a later seance the electrode was gi en a deeper location. Ampicillin was given and he was discharged after about one week. Two week later the patient had a new episode of high fever and persistent infected skin erosion. As it did not seem possible to liminate the Salmonella infection w thout a radical ex ision he wa again hospitalized. On Dec. 30 the pacemaker system was partly removed and a temporary bipolar i lec trode was inserted through the left cephalic ein with the tip implanted in the apex of the right ventricle and connected to an external pacemaker. Blood cultures, now performed for the first time yielded S. typhimurium, hich was also found in the wounds and from the drainage after operation. For this reason the patient was referred to the Department of Infectious Diseases, Roslagstull Hospital.

On admission the patient was afebrile though not antibiotically treated but had some roseola-like efflorescences over the chest. Cultures from blood and wound again revealed growth of S. typhimurium.

T eatment

The patient wa treated for weeks with chloramphenicol, 3.0 g daily and the temperature returned to normal. However 4 days after discontinuing the antibiotic treatment he had a fever spike up to 39.8°C without other ymptoms. Three of 4 blood cultures on te day again showed growth of S. typhimurium. icillin was given 1 eek later 12 g daily. The er subsided within days. After 3 eeks the treat ment was continued orally 6 g daily. This treatment was continued for altogether 3 months.

The intracardiac part of the old cathete had been left in place because of it firm attachment to the endocardium and the risk of damage of the tricuspid valve by its removal. Six weeks after adm ssion it was, however removed without any complication except brief chills. This time blood cultures showed no growth but S. typhimurium was isolated from the intracardiac elec trode tip and from the operation wound. Five weeks later it was obvious that the temporary pacemaker was no longer functioning and the whole system was re moved. The subsequent course was quite uneventful.

Repeated blood stool and urine cultures ere negative for Salmonella, but the Widal titres remained high. Heart volume, as judged by X-ray remained normal. Except for an episode of frequent ventricular extrasystoles or mixed beats on one occasion, repeated ECGs were normal. The patient was discharged 2 months af ter admission, still on oral ampicillin therapy.

During ampicillin treatment the serum concentration of the drug was repeatedly recorded. It was at least 4 μg/ml and thus exceeded the minimum inhibiting concentration for the strain almost tenfold.

Follow-up examination

After discontinuation of the ampicillin treatment the patient was readmitted several times for control the last one 3 years after discharge. The general condition has been excellent. The fever bouts ha e disappeared and no cardiac discomfort has been reported. X-ray of the heart has remained normal, a well as repeated ECGs. Exercise ECG immediately after discharge was normal with normal physical working capacity and there has been no need for a pacemake. Blood cultures have re mained sterile and Widal eaction has given titres as shown in Table I. The first test was performed in another laboratory than the following ones. The result, with different values, was noted immediately after incubation and after standing in the refrigator overnight.

DISCUSSION

The Salmonella infection in our patient may have been of recent origin acquired in hospital or contracted during a trip abroad in the last decade al though no enteric or other symptoms compatible with an acute salmonellosis occurred. It is also possible that the infection started as early as in 1935 when the patient suffered from an undoubtedly epidemic febrile disease. The fever periods recurring ever since 1935 but disappearing after prolonged antibiotic therapy and surgical treat ment are in agreement with this theory. Another support for this theory might be the slow decrease of the high agglutination titres against O and H antigens which indicate an infection of very long duration.

Long-stand ng Salmonella infection has previously been reported by Bottiger and Lagerlöf (4). Protracted courses of salmonellosis believed to be due to a decrease of the activity of the reticuloendothelial system in Schistosoma mansoni infection have also been observed (10, 13). Chronic salmonellosis in a patient with impaired immune de fence function has been described by Stites et al (19). In our patient as well as in Böttiger and Lagerlöf's there were no factors known to interfere with resistance to bacterial infection. Infection may take place at the pacemaker implantation but may also be endogenous as reported by Schowengerdt et al. (16). It is possible that the insertion of the intracardiac electrode in our patient con verted a latent chronic infection with repeated febrile episodes into a manifest septicemia.

No foci were found in the gastrointestinal or

Table I *Bacteriological and serological findings surgical interventions and antibiotic treatment*
B=blood, W=wound D=drainage El=electrode PM=pacemaker E=external I=internal sc.=subcutaneous
Amp.=ampicillin Clox.=cloxacillin, Chlor=chloramphenicol

	Growth of S typhimurium				Widal test*				
	B	W	D	El	O	H 1 2	H.	Surgical management	Antibiotic treatment
1968 May									
Aug. 16								EPM	
Aug. 20								IPM Dissection of the sc electrode	Amp
1970 Feb 13									
Oct 27		+						Change of site of PM bo	Amp discontinued
Nov 2					80*	–	80*		
Dec 7									
Dec 10									
Dec 11								Revision of the erosion	Clox
Dec 14		+							Clox. discontinued
Dec. 30	+		+					Dissection of the sc electrode	Amp
1971 Jan. 2		+	+					Temporary bipolar I PM+partial removal of the old PM system	Amp discontinued
Jan. 3									
Jan. 4	+	+			400	50	400		Chlor
Jan 8		+							
Jan 17									
Jan. 18					400	1 600	800		Chlor I
Jan 1	+								
Jan 22	+								
Jan. 26					1 600	400	800		
Jan. 29									
Feb 8					800	1 600	400		Amp
Feb 1					400	200	400		
Feb. 17		+		+					
Feb 23					400	800	200	Removal of the old intra-cardiac electrode	
March 26									
April 16?								Total removal of the PM system	
May 25					800	800	400		Amp disc
1972 Oct. 10					400	200	100		
1973 March 6					400	400	100		
Sept 5					80	80	80		

Inverted values. Questionable values.

urinary tract, nor as judged from the case history and X ray from the biliary tract Unfortunately no cultures were performed from duodenal secretion The possibility that previous ampicillin treatment had prevented the finding of Salmonella in fecal samples cannot be excluded However bacteria may dwell in the regional lymph nodes and be transported to the blood stream, as was suggested by Böttiger and Lagerlöf

An interesting question in this case is the cause of the acute cardiac conduction failure since the patient had no history or signs of previous cardiovascular disease Though signs of acute myocard ti were not observed the conduction defect might have been a sequel of a preexisting myocarditis Bengtsson and Lamberger in their 5-year follow-up study of cases suggestive of acute myocarditis (3) reported cases, though comparatively rare with atrioventricular conduction disturbances after streptococcal infection.

Acute myocarditis may appear in associ... with infectious diseases of various etiolog... However it is a rare complication in Sa... infection other than with S. typhi and ... A or B Saphra and Winter (15) ... clinical manifestations in over 7 ... monella infections in 1945 a... was mentioned In a ce...

lected during the S typhimurium outbreak in Sweden in 1953 (7) ECG abnormalities were recorded in 59 cases at least 15 of them probably due to acute myocarditis In one case ECG changes compatible with heart block were recorded The diagnosis of myocarditis was verified at autopsy in 5 of 11 fatal cases In 1964 Sanders and Mlsanik (14) described a case of acute myocarditis stemming from an overwhelming septic infection with S cholerаesuis and resulting in death due to rupture of the right ventricular wall Bayer et al (1) reported a mitral subvalvular endocarditis with an abscess in the valvular ring, also caused by S choleraesuis

Spontaneous restitution of defective atrioventricular conduction may occur for instance after improvement of coronary perfusion brought about by artificial pacing as seen in cases of myocardial infarction. In our patient 3 years after removal of the pacemaker system there is still no need for artificial pacing as judged by the patient's history and repeated ECGs This may be the result of elimination of the Salmonella infection followed by healing of a supposed myocarditis which had once probably caused the heart block

Treatment with chloramphenicol and later ampicillin, unfortunately only the latter guided by determination both of bacterial sensitivity and anti- concentration, failed to cure the infection as the pacemaker system was not removed the removal of the pacemaker generator and the subcutaneous part of the old intracardiac electrode was ineffective As elimination of an infection is unlikely in the presence of an implanted foreign body (11 18) we found it mandatory in spite of the risk involved (17) to remove the intracardiac part of the old catheter The removal was justified when it was found that the catheter and the operation wound harboured Salmonella and more so when a definite cure and elimination of the heart block were achieved with no subsequent need for a pacemaker

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THE FUNCTIONAL PATTERN OF THE CADAVERIC KIDNEY IN THE EARLY POSTTRANSPLANT PERIOD

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Abstract. Among series of 96 cadaveric renal transplant recipients the graft function has been studied in 25 grafts characterized by good initial function creatinine clearances varying from 8 to 65 ml/min during the first postoperative day. Initial function did not correlate to the donor's age or to the length of warm and cold ischemia time. Mean graft function as measured by 125I-iothalamate and 131I-hippuran clearances during the period studied was significantly lower than that in recipients of kidneys from living donors whose ischemia times were short and well defined. During the period studied the graft function increased independently of donor's age and length of ischemia time. In connection with ischemia and rejection changes were seen in the functional pattern, with significant reduction in filtration fraction.

In a previous study (7) we described functional patterns in renal transplants from living donors. Various factors differentiate the above situation from that seen in cadaveric transplants. The ischemic period in the latter is much longer and several aspects of the donor's condition may influence the renal function (2, 3, 9, 15). In addition histocompatibility is usually poorer than in living donor transplantation (14).

It is well known that a minority of transplanted cadaveric kidneys resume function immediately after transplantation. The present study was limited to cadaveric kidneys functioning immediately after reestablishment of blood supply to the kidney.

MATERIAL

During the period of study, April 1969–June 1971, 123 transplants were performed in 112 patients. Twenty-seven transplants came from living donors and 96 were cadaveric kidneys. In 25 cadaveric kidneys (26%) the initial function was good and these were included in the present study. Donors varied in age from 10 to 57 years (Table I). Twenty-two donors died of cerebral lesions caused by trauma, bleeding aneurisms or vascular occlusion. In two cases cranial trauma was accompanied by multiple traumatic lesions. Among the remaining three donors two died of brain tumors and one of herpes simplex encephalitis. All donors had been hospitalized for at least 4 hours before nephrectomy, most of them in a neurosurgical ward. In 22 cases information on BP was available during the last 4 hours before nephrectomy. In three the BP was at times 60 mmHg and varied between 60 and 100 mmHg. In the remaining 19 BPs less than 70 mmHg were not registered. All had normal serum creatinine before transplantation and, in 22, urinary production and normal urinary sediment were retained up to the point of nephrectomy. In three cases information is not available on urinary production. Data with regard to the use of pressor agents are incomplete. In most cases perfusion with Perfudex was employed. Mechanical perfusion was not used.

The study material included 24 recipients, 13 men and 11 women, aged — 59 years (Table I). In one patient (T 153) two transplants were performed with an interval of 6 months. Six patients were hypertensive before transplantation and 6 received antihypertensive therapy (Table I). Before or in connection with transplantation bilateral nephrectomy was carried out in three patients (T 118, 156 and 178) and unilateral nephrectomy in two (T 141 and 154). The remaining patients retained their own kidneys in situ during the study. In addition to the two bilateral nephrectomized patients, three patients were anuric. Among the remaining 18 six had creatinine clearance between 3 and 5 ml/min. Additional 12 patients had creatinine clearances less than 3 ml/min. Sixteen of the 24 patients were in regular hemodialysis twice 10 hours weekly before transplantation.

Just after establishment of vascular anastomose 200 mg mannitol were infused. Urinary production was observed immediately and graft function, as determined by creatinine clearance, varied from 8 to 65 ml/min during the first day (Table I). Immunosuppression consisted of azathioprine and prednisone. Immediately before transplantation azathioprine was given in a dose

Table 1 *Data on the donors and recipients*

C_{Cr} = creatinine clearance C_{io} = [125]iothalamate clearance C_{OH_1} = [131]I-hippuran clearance FP = filtration fraction

Donors			Recipients		Pretransplant				Posttransplant (day 0–1)					
Age (y)	Ischemia time (min) Warm	Cold	T no	Age (y)	C_{Cr} (ml/min)	Dia-ly-sis	BP	Anti-hyper-tensive treat-ment	Protein-uria (g/24 h)	C_{io} (ml/min)	C_{Cr} (ml/min)	C_{OH_1} (ml/min)	FP C_{io}/C_{OH_1}	BP
4	77	145	73	36	0	+	160/100	+	–1	32	37	54	12 6	215/120
52	18	102	83	43	1	+	180/100	–	1 3	15	15	116	1_6	165/100
44	15	255	96	39	0	+	180/100	–	1 3	21	35	227	9.3	200/105
30	58	178	101	48	1	+	130/80	–	3.9	19	25	163	11 7	130/100
34	20	144	107	53	2 3	+	150/80	–	4 7	35	39	342	10.2	170/100
26	20	366	105	28	1	+	165/85	–	2.0	44	58	362	12.2	150/80
10	25	332	111	49	3	+	140/70	+	2.5	19	19	104	18.3	160/100
48	35	427	112	46		+	160/130	+	4 0	9	12	31	17.6	175/135
47	24	430	118	50	0	+	170/90	–	3.8	39	43	259	15 0	170/90
37	76	4_4	125	56	2	–	190/120	+	3 1	13	13	68	19 1	180/100
18	19	280	135	47	2–3	–	150/100	–	11 4	8	9	20	40.0	160/105
12	84	48	137	56	2 3	+	140/80	–	12 0	59	65	465	22.1	160/105
48	67	499	138	54	1		130/80	–	6 0	14	17	102	13 7	130/70
48	79	344	140	45	–3	–	140/80	–	0 9	1	24	345	6 1	140/80
23	44	48	141	4	3–4	–	150/100	–	1 6	11	13	68	16.2	180/100
28	14	300	143	5	3–4	–	180/110	–	7	50	56	298	16.8	170/100
16	0	402	149	42	3–4		180/90	–	1 2	18	21	167	10 8	200/100
15	10	647	153a	32	4–5	–	140/80	–	4 9	18	77	186	9 7	140/85
22	43	650	153b	32	4–5	–	150/95	–	7 0	12	14	81	14 8	145/95
49	30	155	154	47	0	+	150/90	–	1 1	6	8	45	13.3	140/100
19	5	401	156	22	0	+	140/70	–	1 7	27	30	41	11 2	170/110
45	6	409	161	59	3–4	–	160/90	–	2.6	6	9	45	13 3	170/110
38	3	787	172	46	3–4	–	150/90	+	7 7	16	18	96	16.7	170/90
51	35	182	178	33	0	+	130/70	–	0 7	41	48	453	16	110/60
39	10	641	185	53	1 2	+	150/95	+	6.4	35	46	361	13 4	170/110
32	27 9	351		43	Average		153/91		4 0	23.5	28.1	177	14 9	163/96
14	22.4	181		11	S D		18/15		3 1	14.5	16.8	107	6.3	24/16
3	4 5	36		2	S E M		3 5/3 0		0 6	2.9	3.4	21	1_	4.8/3 1

5 mg/kg b.w.t. and thereafter 1-3 mg/kg/day dependent on WBC. Prednisone therapy was initiated with a high initial dosage. The first 1 patients (T 73–T 137) were initially given 250 mg/day the remaining patients (T 138–T 185) 150 mg/day. Dosage was reduced during the course of 3–4 week to 30–40 mg/day. I five transplantations (T 135 140 153 +b and 156) severe acute rejection episodes were seen and grafts were destroyed in T 135 and T 153 a+b. The postoperative course was complicated by severe infections with lethal termination in T 125 and T 138. T 161 became anuric during the second posttransplant day and graft anuria lasted for 16 days. In 16 patients the postoperative course was without complications.

METHODS

In 4 recipients simultaneous inulin [125]Iothalamate creatinine PAH and [131]I-hippuran clearances were determined 1 2 hours postoperatively (4 17). All patients received parenteral fluids (0.85% NaCl 100–250 ml/h). At the time of the first determinations all patients had ureteral catheters through which graft urine was collected. Thereafter clearance studies were performed within 2–6 days and again 7–20 days post transplant. A attempt was made to relate graft function to donor' age, warm cold and total ischemia times. Changes in functional pattern were followed during the first 20 posttransplant days.

For calculation of p-values Spearman test was used together with Wilcoxon s rank sum test.

RESULTS

As shown in Table I initial graft function was independent of donor s age. In 13 recipients with kidneys from donors less than 3. years of age the mean [125]Iothalamate clearance was 24 9 ml/min which is not different from the mean [125]Iothalamate clearance for the whole study group. Furthermore there was no correlation between donor's age and level of initial graft function (Table I).

Warm ischemia time i.e. length of time from cessation of circulation to start of perfusion of the isolated kidney varied from 0 to 84 min. The cold ischemia time i.e. length of time from start of perfusion to reestablishment of renal circulation varied from 102 to 787 min. In 17 recipients with a warm ischemia time less than 30 min the mean initial [125]Iothalamate clearance was 23 4 ml/min a figure similar to that of the total study material. Correlation between initial graft function and length of warm ischemia time could not be demonstrated during the interval of 0–84 min. Neither could length of cold ischemia time be correlated to initial graft function.

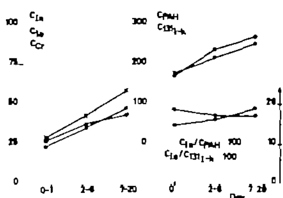


Fig. 1. Mean GFR (ml/min) ERPF (ml/min) and FF values obtained during the first 20 postoperative days in recipient of cadaveric renal transplants.

In 17 grafts with warm ischemia time less than 30 min the cold ischemia time was longer than that in the remaining 8 grafts with a warm ischemia time longer than 30 min. The mean cold ischemia time was 395 and 760 min respectively. This difference is however not significant. The sum of the warm and cold ischemia times did not correlate to initial graft function.

Graft function expressed as mean [125]Iothalamate and [131]I-hippuran clearances increased during the course of the first 6 posttransplant days by 50 and 25% respectively and during the following 14 days by 35 and 15% (Tables I II, III and Fig. 1). PAH and [131]I-hippuran clearances were identical at the first measurement whereas [131]I-hippuran clearance during the following two periods of measurement was somewhat lower than PAH clearance. All measurements revealed good correlation between PAH and [131]I-hippuran clearances ($p<0.001$). Serum albumin at three measurements was 3.34 3.28 and 3.63 g/100 ml respectively. Proteinuria varied markedly irrespective of donor s age and ischemia time. The mean BP level varied only slightly during the study period. The average diastolic BP increased 7 mmHg in relation to values before transplantation. Creatinine clearance values at the time of the three measurements were 20–25% higher than [125]Iothalamate clearance albeit but good correlation was found ($p<0.001$).

The filtration fraction (FF) at the time of the first measurement immediately after transplantation was significantly reduced (13) 14.9% being found. In the following measurements FF n-

Table II *Kidney function on days 2–6*
Abbreviations a in Table I

T no	Proteinuria (g/24 h)	C_{in} (ml/min)	C_{cr} (ml/min)	C_{131} (ml/min)	FF C_{in}/C_{131}	BP
83	0 5	26	26	159	16 4	155/95
96	0.5	37	49	69	13 8	190/100
101	2.4	29	40	184	15 8	140/90
102	2 6	54	77	448	1_1	190/110
105	1 3	64	84	506	12.6	125/80
111	1 6	28	33	194	14 4	150/90
112	3 2	16	70	103	15.5	160/135
118	1 0	64	91	356	18.0	170/90
125	2.7	8	12	58	13.8	190/110
135	3 7	1	77	128	16 4	160/80
137	1 9	60	67	306	19 6	140/90
138	3_	38	43	164	_3_	140/90
140	0 7	1	16	179	9.3	160/90
141	1 6	15	19	89	16.9	10/120
143	0 7	64	71	370	20 0	180/120
149	1 0	43	44	222	19 4	155/105
153a	9	26	37	159	16 4	135/90
153b	1 7	17	22	129	13 2	140/80
154	0.5	15	20	84	17 9	180/110
156	0 7	28	38	264	10 6	190/100
172	3 1	16	24	114	14 0	170/90
178	0 9	66	67	310	1.3	130/70
185	1 4	63	71	287	22.0	170/90
Average	1 7	35_	43 4	17	16 2	16_/97
S D	1 0	20 1	4 1	119	3 6	23/15
S E M	0_	4	5 0	_5	0 8	4.8/3_

Table III *Kidney function on days 7–20*
[illegible]ation as Table I

T no	Proteinuria (g/24 h)	C_{in} (ml/min)	C_{cr} (ml/min)	C_{131} (ml/min)	FF C_{in}/C_{131}	BP
75	0.3	97	92	370	4 9	180/115
83	0 0	46	46	776	16 7	150/95
96	0 0	39	54	288	13.5	160/90
101	0.5	41	57	237	17.3	170/110
102	1 2	77	76	368	20 9	180/100
105	0 5	51	69	380	13 4	160/100
111	0 6	49	69	260	18.8	160/80
112	4 5	26	41	119	21.8	185/120
118	0.8	82	96	374	21 9	165/100
125	1.2	14	21	76	18.4	180/130
137	1.8	63	66	394	16 0	140/80
140	0.0	22	28	146	15 1	140/90
141	1 0	22	27	114	19 1	180/115
143	0 0	63	87	309	20 4	175/110
154	0 0	40	65	233	17.2	150/75
156	0 0	53	66	315	16.8	180/80
172	0.2	32	50	159	20 1	170/90
178	0 0	65	51	277	23 9	160/85
185	0 0	65	90	267	24.3	170/105
Average	0.7	49.6	60 3	261	19 0	166/98
S.D.	1 1	21 6	22.3	99	3 4	14/15
S.E.M.	0.2	5 0	5 1	23	0.8	3.2/3.5

creased showing significantly different values from first to third measurement ($p<0.001$) In the five cases in which severe rejection was seen 5–8 days after transplantation, the FF during days -6 was less than the mean for the whole group namely 13.. x compared to 16.2 %

DISCUSSION

Twenty four patients who had rece ed 25 cadav eric kidneys were studied All demonstrated an initial graft function which expressed as creatinine clearance was greater than 8 ml/min An evaluation was made of graft function in relation to the age of the donor and the length of the ischemia time In addition the functional pattern was de scribed

I recipients of cadaveric transplants it is difficult to evaluate which factors are of greatest significance for graft function. The dono s condition before death aries and is ften unknown and the length of the warm ischemia time is difficult or impossible to determine precisely

We found no relationship between donor s age and initial graft function Neither was there any correlation between graft function and warm or cold ischemia times It has previously been show (3) that, if the donor has not been hypotensive (BP <80 mmHg for >1 hour) and the warm ische mia time is not longer than 130 m urine produc tion will be seen in all transplants within the first 4 postoperative days It has also been shown that the length of the warm ischemia time if <40 min (15) does not influence graft survival

All donors had been hospitalized for at least 4 hours and BP and urinary production values were known for 2.. donors The donors conditions before death and the lengths of warm ischemia times were also relatively well characterized in the present study

Results obtained in recipients of cadaveric trans plants were compared with a group of recipients receiving kidneys from living donors (7) The ages of cadaveric kidney donors were significantly less than those of living donors Ischemia times in transplants from living donors were short and w ll defined (7) At all measurements of graft function in cadaveric transplants as expressed by ¹²⁵Io-thalamate and ¹³¹I-hippuran clearances significantly lower values were found than in recipient of kidneys from living donors the glomerular filtration rate (GFR) increased during the period studied whereas the effective renal plasma flow (ERPF) remained almost constant Also in the present study the ERPF increased but less than the GFR The prolonged discontinuance of renal blood supply thus caused damage both to glomerular and tubular function, which did not disappear during the first 20 days after transplantation

During the study period the graft function increased independently of the donor s age and the warm and cold ischemia times. This is in agree ment w th the observation that compensatory hypertrophy is independent of age (12) In contrast Ogden (11) found after unilateral nephrectomy in living donors an inverse correlation between donor s age and compensatory hypertrophy of the remaining kidney A similar inverse correlation be tween donor s age and compensatory hypertrophy in recipients could not be hown

The FF ¹²⁵Io/¹³¹I-h was significantly lower than normal values (13) in all measurements but was identical to the values obtained in recipients of transplants from l ving donors (7) FF at the time of the first measurement was significantly lowe than that at the time of the third measurement. This means that the ERPF reached maximal values more rapidly than the GFR. Changes cannot be due to variations in BP or serum albumin concentrations since these values were constant during the whole study period In five patients ery low FF values were seen in connec tion with acute rejection episodes (Table II). Thus changes in functional pattern were demonstrated in connection with ischemia and rejection, a reduc tion in FF being found (Tables I and II) This is also manifest in phase II of the isotope renogram where the speed of accumulation in these situa tions is higher than the speed of elimination (6 8).

This study of initially functioning cadaveric kidneys, for which the ischemia times are long and variable revealed a characteristic change in GFR and ERPF Initial graft function did not correlate to donor s age or variations in warm and cold ischemia times which were maximally 84 and 787 min respectively The mean function during the study period was however significantly lower tha the function in recipients of kidneys from closely related donors for which the ischemia times were much shorter Twenty days post transplant the mean ¹²⁵iothalamate clearances were 74% of the function in living kidney donor

grafts and [131]I-hippuran clearances were 7? ? FF values did not vary significantly in the two study groups

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IMMUNOPATHOLOGICAL CHANGES DURING IMMUNOSUPPRESSIVE TREATMENT OF GLOMERULONEPHRITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract Fluorescent microscopy examination of up to 4 consecutive renal biopsies has been performed during combined therapy with azathioprine and prednisone in each of 7 patients with SLE and glomerulonephritis. The changes in the immunopathological picture after treatment showed good correlation to the changes in renal function In 2 cases progression f immunofluorescent changes corresponded well to progression of renal insufficiency The pattern of immunofluorescence microscopy and renal function was unchanged in other cases. In 2 cases an improvement in the immunopathological findings corresponded with an improvement in renal function One patient died shortly after the commencement of the treatment. Progression was seen particularly in the cases in whom the initial immunofluorescence pattern was very lumpy In contrast the immunosuppressive therapy had the best effect in the cases with focal or diffuse granular immunofluorescence patterns.

The development of lupus nephritis is probably related to an immunological disorder and the disease represents a form of immune complex induced glomerulonephritis (6 11 1 16 17 18) Immunoglobulin nd complement are deposited in the renal glomeruli In severe cases similar deposits are found also in the extraglomerular tissue The immunoglobulins and complement are usually located in identical sites and indicate immune complexes (6 16) The pattern and severity of immune complex deposition in immune complex nephritis is correlated to the degree of nephritis (8) Diffuse lumpy or diffuse granular deposits along the glomerular capillaries of all or most lobules are found in severe cases, while diffuse mesangial or focal immune complexes are seen mild disease (11 1) Focal granular deposits are also seen in the early stages of lupus nephritis (1)

Cytotoxic drugs with immunosuppressive effect such as purine antagonist azathioprine (Imurel®) and the alkylating agent cyclophosphamide (Sendoxan) have been tried in SLE-nephritis However the effect has not been correlated extensively to renal function and immunopathological changes This report describes the changes in the renal immunopathological picture d ring combined Imurel and prednisone treatment in 7 patients with lupu nephritis.

MATERIAL AND METHODS

Patients

The 7 patients 4 females and 3 males, all fulfilled the ARA criteria for SLE. All had positive tests for antinuclear antibodies detected by the LE factor test or ANF test The renal function was determined by inulin and PAH clearances, examined few days prior to the treatment and repeated after 3–27 months, usually at intervals of about 6 months as described previously (4)

Biopsies

Consecutive needle biopsies were draw as reported earlier (3) A biopsy was lways taken before combined therapy was initiated and up to 3 sequential biopsies were xamined during the treatment period They were obtained simultaneously with the renal function studies The specimen was at once embedded in Tissue-tec OCT Compound (Ames Co.) quick-frozen in dry ice/acetone and isopenthane and if not examined immediately OCT blocks were stored at −70°C. Cryostat sections were cut approximately 4 μ thick, mounted on glass slides fixed in cold acetone for 10 min, washed and stained with fluorescein isothiocyanate or lissamine rhodamine B-labelled antibody globulin as previously described (20) The methods used for UV microscopy and microphotography have been published lsewhere (20).

Table I. *Changes in immunopathology compared with conventional histology and renal function after combined immunosuppressive treatment of SLE-glomerular nephritis*

II = definite improvement, I = slight improvement, U = unchanged, P = progression

Pat. no.	Age (y.)	Sex	Treatment period (mo.)	Consecutive biopsies examined	Changes after treatment: Immunofluorescence	Light microscopy	Renal function	Initial immunofluorescence pattern
1	25	♂	1/4	Autopsy				Diffuse lumpy
2	34	♀	8	3	P	P	P	Diffuse lumpy
3	28	♀	25	4	I	I (focal)	II	Diffuse granular*
4	1	♀	19	3	P	P	P	Diffuse lumpy
5	5	♂	77	4	II	U (focal)	I	Diffuse granular
6	51	♀	—	3	U	P (focal)	U	Focal granular
7	45	♂	3		U	Unsuccessful		Diffuse granular

* Very fine granules.

Antisera

Antisera to the immunoglobulin classes IgG, IgA and IgM, the complement component C1q and C3, fibrinogen and albumin were made by rabbit immunization using isolated protein in complete Freund' adjuvant (19).

Antibody γ-globulin was isolated, labelled by fluorochrome (*FITC or rhodamine*) absorbed and tested for specificity with sensitive hemagglutination or fluorescein labelling techniques as reported in previous publications (20).

Human sera

Sera were collected from patients simultaneously with performance of biopsies and stored at −20°C. For complement examination fresh sera were kept frozen at −70°C.

Antinuclear antibodies and nuclear antigens

For indirect LE test on serum the method of Larsen and Boyum (13) was used. Indirect ANF test was carried out with mouse liver cell nuclei as antigen (20). Antibodies to native and heat-denatured DNA were detected by immunodiffusion as described by Koffler et al. (11). Nuclear antigens in tissue were traced by using fluorescein-labelled isolated antibody IgG from a patient with precipitating anti-DNA antibodies.

Complement

The complement component C3 was quantitated in serum by Mancini technique with precipitating rabbit antibodies (15). C3 in a normal serum pool was used as standard. For titration of the complement component C1q 0.1 M Na₄ EDTA in 0.025 M Na barbitone buffer was added to a part of the serum in a dilution of 1:10. The serum titre of C1q was obtained by latex immunoglobulin flocculation (10).

RESULTS

Immunological findings prior to combined therapy

Prior to the combined treatment all kidneys contained diffuse lumpy or granular deposits or in one case focal granular deposits of immunoglobulin and complement in the glomeruli. The initial immunofluorescent patterns of immunoglobulins and complement in the glomeruli are shown in Table I. The granular deposits in cases 3 and 5 were seen as regular beads on a string along the glomerular capillary walls (Fig. 1). In patient 3 the granules were smaller than usual and the fluorescence relatively weak. In case 7 the granules varied in size and shape and were confluent in smaller areas. However, the pattern was recorded as lumpy only when granules flowed together into larger homogenous masses. In the cases with focal alterations several glomeruli were found without fluorescence and the affected glomeruli had most often lobular deposits of immunoglobulins and complement.

In addition to the major deposits of immunoglobulins and complement factors in the glomeruli, immune complexes were also detected in the walls of extraglomerular vessels in two patients (nos. 1 and 4) in the tubulus cells, tubular basement membranes and throughout the interstitial connective tissue. Albumin was detected only in collections of small droplets intracellularly in the proximal tubulus (Fig. 2). Fibrogen, most often as lumpy or granular deposits, was seen in the glomeruli and in the connective tissue only in the most severe cases. In a few instances isolated deposits of complement C3 without immunoglobulin were detected, particularly in Bowman's capsule and along the tubular basement membrane (Fig. 3). The distribution of the initial localization of proteins in the different structures of the renal tissues is shown in Table II.



Fig. 1. Kidney biopsy from patient 5. Granular deposit seen as "beads on string" along the glomerular capillary wall (stained with FITC-conjugated anti-C3). Similar staining was obtained with anti-IgG.



Fig. 2. Kidney biopsy from patient 7. Collections of small droplets of albumin intracellularly in the proximal tubuli (stained with FITC-labelled anti-albumin).

In 6 cases the immune complexes contained IgG, IgM and C3. In one of them (no. 4) immune complexes with IgA appeared later during therapy, and in 3 (nos. 2, 5 and 7) only traces of IgM were present. In one case (no. 6) there was a strong immunofluorescence with anti-C3, while only traces of IgG and no IgM were present. In patient 4 the glomeruli also contained large globular bodies with nuclear antigens detected by FITC-labelled anti-DNA. In addition to immunoglobulins and complements C3 and Clq. These probably represented hematoxylin bodies.

Immunological findings after combined therapy

In Table I the degree of immune complex deposition in the glomeruli after treatment is compared to changes in renal function. Improvement or progression was determined the following way: Improvement was recorded when there was a decrease in the amount of deposits or intensity of immunofluorescence. A change from diffuse to focal distribution of immune complexes in glomeruli was also taken as an indication of improvement (Table III). A further sign of improvement immunologically was change in the immunoglobulin class of immune complexes from IgG to IgM (Table III). Such an alteration was not absolute, but a relative change could clearly be observed in some of the cases. A progression of the condition was measured by an increase in the amount of deposition, a more diffuse distribution, and a change towards more IgG in the immune complexes.

When these criteria were utilized to measure improvement or progression, the immunopathological findings corresponded well to the changes function (Table I). During the course



Fig 3 Kidney biopsy from patient 4 Isolated deposits of complement factor C3 w thout immunoglobulin along e tubular basement membrane (stained with FITC lled anti-C3)

a clear improvement was detected n cases (nos 3 and 5) by a reduction of the immune complexes (Fig 4) This was most marked in patient 5 who was followed for more than years with 4 consecutive biopsies, with a steady decrease in immune complex deposition and a change to more IgM deposits The same trend was pronounced but less marked in patient 3 In both these patients a definite clinical improvement was observed most marked in patient 5 but also definite in patient 3 The tendency to improvement was also observed in light microscopy of consecutive biopsies from these 2 patients Table I also shows that in both these cases the light microscopy changes were described as focal while the immunological appearance was that of diffusely localized immune complexes

A slight progression was observed in cases (nos and 4) They had heavy lumpy deposits in all glomeruli prior to the combined treatment (Fig 5) There was clearly a progression in the immunoglobulin depositions and a change to heavy lumpy IgG deposits with less IgM (Table IV) In both these cases the progression was clearly associated with a progression in the renal function and in light microscopic changes (Table I) In one patient however a partial improvement occurred later (Table IV)

In cases (nos. 6 and 7) the immunopathological picture was almost unchanged One of them was followed only for a short period while the other with focal granular deposits was followed for — months In both these patients also the renal function was almost unaltered

Relationship to other immunological findings

All the 7 patients had positive tests for antinuclear antibodies at the initial stages of the disease In patient 4 there was a marked intensification of the immunopathological findings at one stage during the study After 9 months of therapy one biopsy showed a large amount of immune complexes

Table II *Prote n deposition before treatment in renal tissue from 7 ases with SLE glomerulonephritis*

Protein	Glomeruli	Bowman capsule	Proximal tubuli	Juxta-glomerular vessels	Interstitial tissue
IgG	7*		4		
IgA	1	0	0	0	0
IgM	6*	1		1	1
C3	7		4	3	
Albumin	0	0	5	0	0
Fibrinogen	3	0	1	1	

One had only traces.
Three had only traces.

Table III *Glomerular deposits observed by immunofluorescent microscopy of renal biopsies from patient 5*

Trace amounts within parentheses

Combined therapy (mo.)	Immuno-fluorescence pattern	Glomerular deposits Quality	Intensity	Quantity
0	Diffuse granular	IgG/(IgM)/C3	+++	+++
6	Diffuse granular	IgG/IgM/C3	++	++
13	Focal granular	IgG/IgM/C3	++	+
27	Focal granular	(IgG)/IgM/C3	++	+

deposited in the interstitial connective tissue in vessel walls and in the proximal tubulus A renewed biopsy 10 months later showed a decline in these extraglomerular complexes This improvement was followed by regress of the cellular infiltration The transitory exacerbation in this patient corresponded also to a drop in the serum complement and a rise in the serum antibody titres to nati e and heat-denatured DNA. ANF and LE factors were present in high titres. However after 19 months of combined therapy ANF and LE factors were detected in significantly lower titres than before treatment and anti-DNA antibodies could not be demonstrated The serum complement was normalized Heavy lumpy deposits were however still present in all glomeruli but the intensity of fluorescence was somewhat weaker (Table IV) Also the renal function of this patient stopped to progress after this long treatment period

In another patient (no 5), who was followed for 27 months the steady improvement in immunofluorescence findings in kidney biopsies together with an improvement in renal function was also seen as a drop in serum antibody to native and heat-denatured DNA Furthermore the complement values were normalized

Patient 1 was examined only by an autopsy specimen after 1 week of combined therapy He had progressive nephritis and died of renal failure shortly after the first symptoms On the day prior to death the serum complement levels were extremely low The serum anti-DNA antibodies were initially very high-titred but decreased simultaneously with the serum complement probably due to their blocking in immune complexes This corresponded to the immunofluorescent findings in the renal tissue that both glomeruli and the total cortex tissue were crammed with immune complex IgG and C3 aggregates

DISCUSSION

Treatment with cytotoxic drugs such as purine antagonists and alkylating agents in combination with prednisone has been shown to improve the clinical course of lupus nephritis (4 5 9) However no definite correspondence between clinical improvement measured by renal function studies, and immunosuppressive effect, measured by immunological methods has been recorded For this reason it has been suggested that these drugs act on non-immunological mediators (7). In the present

Table IV *Deposits observed by immunofluoresce t micro copy of renal biop ies from patient 4*

Trace amounts within parentheses

Combined therapy (mo)	Pattern	Glomerular deposits Quality	Intensity	Quantity	Extraglomerular deposits (tubular/interstitial) Quality	Intensity	Quantity
0	Diffuse lumpy	IgG/IgM/C3	+++	++	IgG/C3	+	(+)
9	Diffuse lumpy and granular	IgG/IgM/IgA/C3	++++	+++	IgG/IgM/C3	++++	++
19	Diffuse lumpy	IgG/IgM/(IgA)/C3	++	++	IgG/IgM/C3	++	++













Fig 3 Sections from the kidney biopsies of patient 4. Before treatment there were diffuse lumpy deposits as detected by FITC-conjugated anti-C3 (*a*) and anti-IgG (*b*). During therapy the lumpy deposits remained and partly progressed (*c*) 9 months after initiation of the therapy

study however during the treatment with combined immunosuppressive drugs there was de finite decline in the glomerular immune complex deposition in two cases This effect clearly cor responded with an improvement in the renal func tion and the clinical course During the therapy it was also shown that the pattern of immunoglobulin and complement deposition could change from dif fuse granular to focal Furthermore in these cases there was a tendency to shift from IgG to IgM deposition of glomerular immune complexes. This is in accordance with other findings of changes from IgG to IgM, when a positive effect of immunosuppression is observed (2 14 22)



Fig 4 Kidney biopsy from patient 5 had initially extensive diffuse granular deposits Staining both with FITC labelled anti-C3 and anti-IgG (*a* and *b*). After 77 months of therapy the deposit was changed to focal granular staining with anti-IgG and anti-C3 Fig show some staining with anti-IgG, while another glomerulus is almost unstained (*d*).

The two cases in whom the best improvement was seen were not among the most severely affected. In contrast, the two patients with the most diffusely localized lumpy deposits (nos. 2 and 4) did not show any improvement but a further progression of the immunopathological findings, also related to a progression of the renal failure. Even in these cases some effect on the active deposition of immune complexes could not be ruled out. The absent clinical effect might be due to irreversible tissue damage. However, in patient 4 a small improvement was initiated after long treatment, indicating that some improvement could be achieved in such cases. Our findings also supported the assumption that patients with diffuse lumpy deposits or irregular large granules had the most severe nephritis with pronounced renal failure. The patients with small and regular non-confluent granules were clearly those who had as a rule better renal function and responded most satisfactorily to the combined treatment. It thus appeared that in these stages the immune complex depositions were more easily reversible, in contrast to the late and terminal stages where functional tests and light microscopic changes deteriorated unrelated to the immunopathological picture. This can also explain why the renal function in patient 4 progressed while at one stage there was a slight regression of immune complexes.

The immunopathological findings and the renal functional studies were also compared with the findings of light microscopy (Øystese et al., in prep.). This showed mostly a corresponding improvement or progression to that observed by immunofluorescent and renal function studies. However, in one case there was an unchanged light microscopy picture while the immunofluorescence and renal function studies showed a marked improvement (no. 5), whereas in another case (no. 6) there was a slight progression of the light microscopy findings.

In lupus nephritis immunofluorescent examination of renal biopsies can give information about the cases in which a favourable effect of immunosuppressive therapy can be expected. It is therefore possible that this method can be used for selection of patients for therapy. In addition, it is useful to follow the therapeutic results, particularly in the early disease stages. If the treatment can be started before the deposits have become irregular, confluent or lumpy, the result is assumed to be better. In addition to immunofluorescent examination of biopsies, the effect of the treatment should also be followed by quantitation of anti-DNA antibodies, complement components, immune complexes and cryoglobulins in the patient's serum (11).

The findings indicate clearly the importance of immunofluorescence examination of kidney biopsies at an early stage of lupus nephritis. If combined therapy is initiated before irreversible changes have occurred, a different course of the disease with a longer survival could be expected.

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BLOOD PRESSURE DEVELOPMENT AND CHARACTERISTICS OF SUBJECTS WITH MODERATE BLOOD PRESSURE ELEVATION

A T 'o-year Follow-up Study in a Random Population Sample

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Abstract. In an ongoing multifactor primary preventive trial in Göteborg, Sweden one third of all men born in 1918–19 were invited to screening examination including history-taking and blood pressure (BP) and heart rate (HR) measurement. The screening examination was attended by 1427 men (76%). Those who were considered to have borderline BP L SBP 160–175 or DBP 95–115 mmHg, were reexamined two years later regarding BP and HR. Of these 493 subjects 12% were considered hypertensives, i.e. they had SBP>175 or DBP>115 mmHg or had been put on antihypertensive treatment. If the majority of new cases of hypertension is assumed to come from the borderline group, the incidence of new hypertension in thi age (52 y) is about 2% At the reexamination 39% of the former borderline hypertensives had normal BP A substantial part of those with borderline BP thus had normalized their BP within two years. Those who developed hypertension wer characterized by higher SBP and HR already at the initial screening examination. They also tended to have lower physical activity at work and during leisure and higher stres experience. These differences were, however not statistically significant.

It has repeatedly been shown that hypertension is a condition which cannot be well defined by certain blood pressure (BP) limits. The BP distribution in the general population is unimodal and tailed to the right (1 20). The relatively few cases with secondary hypertension and extensively elevated BP will generally not influence this distribution to any substantial degree The unimodal BP distribution fits the hypothesis that the majority of cases with high BP ha e essential hypertension i.e the cause is not a single organ damage Most findings in man, supported by the findings in hypertensive rats (8, 10) are in accordance with a liability to hypertension carried by several genes and influenced by environmental factors. The primary disturbance affects the autonomic nervous control. Secondary influences on both the suprarenal renal fluid control and central nervous systems are probably of importance in the later stages of the process.

Today there is fairly general agreement on the necessity of treating cases with definitely elevated BP at least if some cardiovascular complications are found (19) Since the hypertensives usually do not appear at the physician s office because of symptoms the discovery of the disease has to rely upon screening of population groups at risk. When such a screening is performed a group with BP in the borderline zone between normotension and definite hypertension is found It is not known how often BP measurements have to be repeated in the borderline group if an optimal number of cases is to be detected i e. the incidence of definite hypertension within a group of borderline hypertensives is not known. Nor do we know the difference in characteristics between those borderline hypertensives who develop definite hypertension and those who do not

The present study is a part of a multifactor primary preventive trial in which hypertension is treated a randomly selected population sample of Swedish men. The aims were to study the BP development during two years in a randomized male population sample with moderate BP elevation in order to find out: 1) The incidence of new cases of definite hypertension.) Whether there were any special characteristics seen in those cases with moderate hypertension who developed definite hypertension during two years.

STUDY POPULATION AND METHODS

The subjects for this study of moderate BP elevation as will be defined later were recruited from a multifactor primary preventive trial which started in 1970 (20 1) The intervention group consisted of a random third of all men aged 47 55 years living in Göteborg, Sweden. The subject were born in 1915–5 The two thirds who were not examined constituted the control group for the preventi e trial In this report data from men born in 1918–19 mean age 5̃.3 years and be longing to the i tervention group are analysed.

The screening examination began in 1970 with men born in 1915 and ended in 1973 with those born in 1925 The subjects received postal questionnaire concerning heredity for myocardial infarction and stroke their own chest ymptoms, some earlier diseases, present physical activity during work and leisure and smoking habits. Those who answered the questionnaire (84%) were in vited to screening examination which included, among other things, measurement of height, weight BP plasma cholesterol and heart rate (HR) by ECG 76% attended the screening examination

Subjects with systolic BP (SBP) above 175 mmHg or diastolic BP (DBP) above 115 mmHg were referred to a second BP measurement within two weeks. The criteria for further BP determ nations and/or diagnostic x amination were given in previous paper (20) Subjects with screening SBP 160–175 or DBP 95–115 mmHg were reexamined after two years and constitute the material for this study Those with SBP<160 and DBP<95 mmHg were considered as normotensives and had no further BP control until the ordinary work-up in the preventive trial four years after the initial screening.

Of the 1427 men who attended the screening examination 628 had SBP<160 and DBP<95 while 265 had SBP>175 or DBP>115 mmHg. Of those who had SBP 160–175 or DBP 95–115 mmHg 493 participated in the reexamination wherea 41 did not

According to the BP reading *at the reexamination two years after the initial screening* the subjects were divided into three groups: (A) Subjects with SBP>175 or DBP>115 mmHg (n=41) or subjects who had been considered hypertensives during these two years and had received antihypertensi e treatment (n=17). (B) Subjects with SBP 160–175 or DBP 95–115 mmHg (n= 44). (C) Subjects with SBP<160 and DBP<95 mmHg (n 191)

The BP both at the initial screening and at the rescreening, was measured in the afternoon in the right arm with the subject in the sitting position after about 5 min rest. A 12.5 cm wide and 26 cm long arm cuff was connected to a mercury manometer DBP phase 5, when the Korotkoff sound disappeared, was used. Pressures were read to the nearest mmHg. At the initial screening the BPs were measured by one of several physicians at the reexamination by one nurse. HR was calculated from the ECG Body height and weight were measured according to Rose and Blackburn (13).

Physical activity at work and during leisure was

Table I *Classification of physical activity in the postal questionnaire*

Activity during work	Score	Activity during leisure	Score
... work is mainly sedentary and you do ... walk about much during working hours. ...amples of work of this type are desk work, watchmaking and assembly of light components	1	*I active* You spend most of your time reading, watching TV at the cinema or are engaged in other sedentary activities during leisure hours	1
Relatively active You walk about quite lot in your work but do not carry or lift heavy objects. Examples of occupations of this type are mobile office work light ndustrial work, supervisory work instructional work of type involving a lot of walking about and work as a shop assistant		*Some physical activity* You walk cycle or are active in some other way at least 4 hours a week. This includes walking or cycling to and from work as well as Sunday walks, normal gardening fishing, tabletennis or bowling	2
Active+moderat You walk about and lift objects quite a lot or walk up stairs or slopes a lot. Examples are postmen people working in heavy industry carpenters, pipelayers	3	*Regula phy ical activity* You engage in running, swimming tennis, badminton gymnastics or other sports, heavy gardening and the like Note that this means at least 3 hours a week on an average	3
Heavy manual labour You have a heavy manual job lift heavy objects and exert yourself physically Examples are forestry workers, agricultural workers, fishermen using heavy equipment and builders labourers	4	*Regular physical coaching* You engage in hard training and compete in running skiing, swimming, football handball, etc regularly and several times each week	4

Table II *Classification of stress experience according to the postal questionnaire*

By stress is meant a state of mental tenseness, irritability, nervousness, agony or insomnia due to, for example, trouble with your work or at home, etc.

	Score
Have never experienced stress	1
Have experienced occasional periods of stress	
Have experienced occasional periods of stress during the last 5 years	3
Several periods of stress during the last 5 years	4
Constant stress during the last year	5
Constant stress during the last 5 years	6

analysed according to a four-point scale. The four categories were illustrated with drawings in the postal questionnaire, the classification is given in Table I. The scoring of activity was done according to a calculation of energy expenditure (15).

Stress experience was coded according to a six-point scale with increasing number for increasing stress experience. The questions in the questionnaire are given in Table II. Scores 5 and 6, representing constant stress experience during the last one and five years, were combined in the analysis.

In this paper the term "borderline" only indicates BP at the border between normotension and hypertension and is not defined as suggested by Julius and Schork (6).

In the statistical analysis χ^2-analysis was used for comparison of frequencies. Analysis of variance was used for testing the hypothesis that the means of different groups were the same. When this test showed significant differences between means, Sheffe's method was applied to find the location of the differences. The level of significance used was $p = 0.05$.

RESULTS

SBP, DBP and HR at reexamination in the earlier defined BP groups are shown in Table III. HR was highest in those who had definite hypertension and fell significantly from group A to group C ($F = 21.18$).

SBP, DBP and HR at the initial screening examination in normotensives, in the borderline BP groups and in untreated and treated hypertensives are given in Table IV. Borderline hypertensives who had SBP>175 or DBP>115 mmHg at reexamination had significantly higher SBP ($F = 22.59$) and HR ($F = 3.46$) than the other two groups of borderline hypertensives already at the initial screening examination. There was however no

Table III *SBP, DBP and HR for borderline hypertensives at reexamination two years after the initial examination*

BP group at reexamination	SBP $\bar{x}$		DBP $\bar{x}$		HR $\bar{x}$	
A (n=41)ᵃ	177	13	110	10	80	11
B (n=44)	155	10	101	6	77	10
C (n=191)	139	10	88	5	72	9

ᵃ Only untreated subjects.

Table IV *BP and HR at the initial screening examination in relation to values at rescreening*

	At initial screening					
	SBP $\bar{x}$		DBP $\bar{x}$		HR $\bar{x}$	
Normotensives (n=628)	135	[illegible]	86	7	70	[illegible]
Borderline hypertensives at reexamination after years (n=493)	154	12	101	6	75	14
A (n=58)	[illegible]	8	103	7	79	15
B (n=44)	155	111	102	6	74	13
C (n=191)	151	12	100	5	74	14
Definite hypertensives (n=265)	182	19	113	12	80	15
Untreated (n=169)	186	15	114	11	79	14
Treated (n=96)	175	22	111	14	81	15

difference in DBP between the three groups at the initial examination. The mean HR at the initial examination in the whole group of borderline hypertensives ($\bar{x}$=75) fell between those with definite hypertension ($\bar{x}$=79) and the normotensives ($\bar{x}$=70).

The percentage of men with low physical activity at work and during leisure (score 1), constant stress experience (scores 5–6) and the mean body weight for the three borderline BP groups are shown in Table V. No significant differences were found. There was however a tendency to a higher percentage of physically inactive persons at work and during leisure and of stress experience among those with SBP>175 or DBP>115 mmHg at reexamination (group A). The weight was lowest in the group of men whose BP decreased (group C).

The frequency of subjects with myocardial in-

Table V *Lo physical activity at work and during leisure (score 1) constant stress experience (scores 5–6) and body eight in borderline hypertensives grouped according to BP groups at reexamination after two years*

BP group at reexamination	Low physical activity (%) At work	During leisure	Constant stress (%)	B wt (k)	(s)
A (= 58)	35	1	23	82	13
B (n = 44)	27	20	16	81	10
C (n = 191)	29	21	13	78	10

farction or stroke among the parents was also analysed in the different groups but no significant relationships were found Neither were any significant differences found concerning smoking habits

DISCUSSION

In this type of population studies several thousands of subjects have to be screened and a large proportion of them will eventually have to be checked regularly It has therefore within our limited resources only been possible to conduct more special studies in subsamples of the total population screened Such studies are planned or under way

The present study of repeated BP measurements a group of borderline hypertensives over a two-year interval constitutes another type of investigation It gi es some information of interest as it covers a random sample of the total population of males within a limited age range of a well defined ethnic origin and living in a limited and well defined geographic area Thus the findings could not be due to age-related factors or to special selection of cases On the other hand there is a certain drop-out (25%) The non-participation group might contain a somewhat higher rate of hypertensives and other cardiovascular diseases.

The BP was measured only once both at the initial screening and at the reexamination after two years It might be argued that this is not enough to characterize a subject as normotensive borderline" hypertensive or definitely hypertensive. On the other hand the accuracy of using a casual BP reading to characterize groups of individuals has been well established (3 13)

The arbitrarily chosen cut-off points of SBP 175 and DBP 115 mmHg may seem somewhat high They have been chosen as an earlier study showed a rapidly rising frequency of hypertensive organ manifestations above these limits (18) Several factors in the screening and reexamination procedure may account for the fact that the BP distributions are shifted to the right i e to higher values These factors have been discussed previously (20)

Of the 493 subjects who initially had "borderline BP elevation 58 (1 %) could be considered as definite hypertensives two years later From earlier studies we know that the majority of subjects who develop hypertension have had somewhat high pressures already at the first examination (14 16) Whether this is true also for the present population is not known as we have not reexamined the total sample yet The minimum incidence of new hypertension according to these findings is 29 of 1477 per year or about 2% In a study of the natural history of hypertension in a Chicago company the risk of developing sustained hypertension in 25–30 years was calculated to be 25% (14) i.e an incidence of about 1% per year In a study on male Israeli civil service workers the five year incidence rates of hypertension were found to increase with age and were also related to place of birth for the immigrants (7)

It might seem curious that 191 subjects or 39% of those with borderline hypertension had normal BP two years later One important explanation of this is of a purely statistical nature The decrease may be accounted for by regression towards the mean (11) There are however also reports of continuous decrease of BP with time Thus Stewart in a prospective study of 53 young men with mild BP elevation without organ manifestations (17) reported that 12 subjects (23%) had a continuous fall of BP over a period of 6.7 years Another study (2) showed a much lower percentage of BP normalization in subject with "borderline hypertension. These studies do not however reflect the situation in the total population but in selected groups of subjects

It must be stressed that even minor differences in BP measurement, such as time of the d y time of rest before measurement etc make comparisons between different prevalence studies of hypertension very difficult. Hitherto we have no suspicions that the true BP distribution is different

in our city than in other communities from which studies have been published. The rate of definite hypertension and "borderline" hypertension with our cut-off points is in fairly good accordance with some other studies Thus our prevalence of "borderline" hypertension viz. 34% is in good accordance with that found by Jul us and Schork in their review of borderline hypertension (6)

The method used to study physical activity and stress experience, i e postal questionnaire, has the disadvantage of having to rely upon the per so s subjective opinion This study was how ever done prospectively which reduces the risk of bias The finding of a tendency to lower physical activity and higher stress experience among those borderline hypertensives who developed de finite hypertension ought to be checked by studies in other populations and if possible by using more objective methods.

In the present study subjects with definite hypertension had a significantly higher HR than both the normotensives and those with "border line" BP elevation. Earlier too it has been well documented that high BP is positively correlated with HR (2 4 6). Furthermore those borderline hypertensives who developed definite hypertension were characterized by having a higher HR and higher SBP indicating a hyperkinetic circulation Julius et al (5) found this to be due both to an increased sympathetic effect and to a reduced parasympathetic effect in "borderl ne hyper tension. Their findings are in agreement with those in established hypertension published by Korner et al (8). Studies in hypertensive rats have also shown the mportance of increased sympathetic ac tivity for the development of hypertension (10)

It is quite possible that the disturbed cardiovascular regulation with hyperkinetic circula tion is an early event in the disturbed regulation of BP in hypertension and thus characterizes subjects with a BP progression from normotension to definite hypertension. Whether this w ll indicate any special form of treatment suitable for this group calls for separate studies.

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THE RELATION OF PLASMA RENIN ACTIVITY TO LEFT VENTRICULAR HYPERTROPHY AND RETINOPATHY IN PATIENTS WITH ARTERIAL HYPERTENSION

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Abstract Plasma renin activity (PRA) has been determined in 77 hypertensive patients in the supine position at 8 a.m. after 48 hours sodium restriction. Mean PRA was highest in patients with renal disease but individual values overlapped with those from the 51 patients with "essential" hypertension. Ten patients were found to have low and unresponsive PRA (≤0.4 ng ang. I/ml/h); primary aldosteronism was diagnosed and verified by operation in 3 of them. After exclusion of patients with renal disease it was found in the remainder 54 patients, that the presence of left ventricular hypertrophy (LVH) was associated with a higher mean PRA than in patients without LVH. The relative frequency of LVH was however higher in the 10 patients with low unresponsive PRA (50%) than in patients with normal or high PRA (43.3%). LVH was most closely related to the BP level especially the systolic BP which was significantly higher in patients with LVH than in those without. It is concluded that the level of the BP is the single most important factor for the development of LVH while renin–angiotensin are probably of no direct importance. The patients were divided into two groups according to the severity of retinal changes. The relative frequency of the most advanced retinopathy (grades II and III after Keith and Wagener) was, unlike the occurrence of LVH found to be lower in patients with low PRA, occurring in 1 of 10 patients as opposed to 1 of 44 patients with normal or high PRA. Presence of retinopathy grade II–III in patients with normal/high PRA was associated with a significantly higher mean PRA than in patients with grade 0–I changes. Patients with retinopathy grade II–III were not older their hypertension had not been known for a longer time, but they had significantly higher systolic and diastolic BP than patients with retinopathy grade 0–I. Renin-angiotensin may play a direct role for the development of retinal hypertensive arteriolopathy. The close association between high mean PRA and BP in the patients with the most advanced retinopathy could mean that angiotensin increases the adverse effect of high BP on arteriolar walls.

In most patients with essential hypertension the plasma renin concentration (3 4) and the plasma renin activity (PRA) (6 10) are normal. In some patients PRA is elevated and in 20–25% it is subnormal and fails to respond to sodium restriction and upright position (6 10). The different PRA levels in patients with essential hypertension could imply that different pathogenetic mechanisms were operating. However even in renal hypertension the pathogenetic role of renin–angiotensin is not entirely settled (1 7 8 13 14), and its implication in other hypertensive states is in fact more questionable.

Although the relation of the renin–angiotensin system to the development or maintenance of elevated BP is a matter of discussion renin–angiotensin might be of importance for the development of hypertensive cardiovascular complications. This assumption is supported by the fact that in patients with primary aldosteronism and low or undetectable PRA hypertensive retinopathy is mild if present at all and cardiomegaly is seen in only 40% of the patients (9). Remarkable are the observations of Brunner et al (6) that coronary infarction and stroke did not occur in their patients with essential hypertension and low PRA in contrast to the relatively frequent occurrence in their patients with normal or high PRA.

In patients with malignant hypertension both plasma renin concentration angiotensin II and PRA are regularly found to be elevated (4 8 17 20) as is the urinary aldosterone excretion. This activation of the renin–aldosterone axis is thought to be responsible for the accelerated course of the hypertensive disease (7 17 20).

The intention of the present study has been to examine the relation between the level of PRA and the occurrence of left ventricular hypertrophy (LVH) and retinopathy in patients with arterial hypertension.



Fig 1 Individual and mean PRA values in 72 patient w th ntenal hypertension determined in recumbent position for the last 10 hours and after 48 hours sod m restriction (Na<15 mEq/24 h).

PATIENT MATERIAL AND METHODS

the period Jan 1970 to March 1973 7? hypertensive in-patient at Medical Department B Ake Hospital ere extensively evaluated for secondary hypertension. The patient represent a selected group as more than 60% w re referred from other hosp tal or specialists. Only patients w th diastolic BP ≥95 mmHg on repeated measurement were included Patients with malignant hypertension were ex luded The mean age of the patients w s 43 1 years (range 17–67) only three patients were older than 60 years

The mean BP for all patients (182/116) was calculated from the average of three BP readings in each patient These were recorded with the patient recumbent on the third fourth and fifth mornings after admission.

Forty-six patients had been treated with antihyper tensives, all treatment was stopped at least 2 weeks before the study Some patients were referred with suspicion of primary aldosteronism because hypokalemia had developed after thiazide diuretics. They were given potassium supplement, and serum sodium and potassium were within the normal range at the time of the study except in 4 patients who were still slightly hypokalemic.

One intention of the study wa to compare the value of rapid sequence pyelography and isotope renography a screening procedures for renal hypertension Both these examinations performance of renal angiography s well a determination of peripheral vein PRA and renal vein PRA were done in the first 60 patients studied

In the last 12 patients isotope renography alone as used a screening procedure for renal hypertension, as the method described by Norman (23) has been shown to be a reliable screening method for renal hypertension (18).

The peripheral vein PRA alues compared th hypertensive complications in the present communication were measured at 8 m after 48 hours sodium restriction (Na supply <15 mEq/day) with the patient recumbent for the last 10 hours PRA (angiotensin I generation rate) w s determined radioimmunologically (?7) and expressed as ng angiotensin I generated per ml plasma per hour (ng ang I/ml/h).

In all patients with PRA below 0.5 ng ang. I/ml/h and in most others we also examined the PRA response to upright position and an acute oral load with furosemide Primary aldosteronism was diagnosed hen PRA was suppressed and unresponsive together ith the finding of a normal or high secretion rate of aldosterone not suppressed by sodium loading

Th degree of retinopathy was classified from 0 to IV after Keith and Wagener All the fundi were evaluated by the same observer (H J) who at that time did not know the actual value for PRA LVH as diagnosed from ECG adopting the simplified score system described by Skjaeggestad and Kjerulf (25) and from cardiac X-ray LVH was said to be present when $S_{max}+R_{max}$ in V_{1-6} were 40–49 mm either in combination with a entricular activation time in V_{5-6}≥0 05 sec or in combination with radiological signs of cardiac enlargement (heart volume: women ≥450 men ≥500 ml/m²). $S_{max}+R_{max}$ in V_{1-6}≥50 mm were alone regarded as sufficient to diagnose LVH

RESULTS

Among the 72 patients primary aldosteronism was diagnosed and verified by operation in 3 In 18 patients the hypertension was caused by or associated with renal parenchymal disease or renal arterial stenosis (4 were operated upon) In the remaining 51 patients no possible cause for the hypertension was found and their hypertension was classified as essential" The mean PRA in the renal disease group (3 44 ng ang I/ml/h) was significantly higher ($p<0.0025$) than that of the essential hypertension group (1.85 ng ang I/ml/h). The individual PRA values were however over lapping in the two groups as shown in Fig 1 Similarly in some patients with essential hyper tension low and unresponsive PRA was found as in patients with primary aldosteronism

Table I *PRA (ng ang. I/ml/h mean and range) in relation to type of hypertension and to the presence or absence of LVH and the grade of retinopathy in 72 hypertensive patients*

Type of hypertension		LVH absent		LVH present			Grade of retinopathy 0–I		II–III		
			PRA		PRA	*p*		PRA		PRA	*p*
"Essential"	51	28	1.51 (0.[illegible]–4.8)	23	2.27 (0 –6.8)	<0.05	38	1.46 (0.2–4.8)	13	3.0 (0 –6.8)	<0.005
Renal disease	18	8	2.86 (0.9–5.5)	10	3.90 (1.4–5.7)	<0.30	14	3.1 (0.9–5.6)	4	4.55 (3.6–5.7)	<0.20
Primary aldosteronism	3	2	0.[illegible] (0.[illegible]–0.2)	1	0.3		3	0.23 (0.2–0.3)			
Total	72	38	1.72 (0. –5.5)	34	[illegible]69 (0.[illegible]–6.8)	<0.01	55	1.83 (0.2–5.6)	17	3.36 (0 –6.8)	<0.005

In Table I the presence of LVH and the degree of hypertensive retinopathy are related to the mean PRA in the three groups of patients. The presence of LVH in patients with essential hypertension was associated with a significantly higher ($p<0.05$) PRA (2.27 ng ang. I/ml/h) than in patients without LVH (1.51 ng ang. I/ml/h), whereas mean PRA in patients with renal disease and LVH (3.90 ng ang. I/ml/h) was not significantly higher ($p<0.30$) than the value in the "renal" patients without LVH (2.86 ng ang. I/ml/h). For all patients lumped together we found a highly significant difference in mean PRA ($p<0.01$), with the lowest value in the patients without LVH.

The same trend was found for the grade of retinopathy. In the patients with more severe changes (grades II and III) the mean PRA was higher than in patients with ophthalmoscopy grades 0 and I (Table I). The mean PRA values (4.55 versus 3.1 ng ang. I/ml/h) were not significantly different in the small number of patients with renal disease, unlike the respective PRA values for the patients with essential hypertension (3.0 versus 1.46 ng ang. I/ml/h) and for all the patients lumped together (3.36 versus 1.83 ng ang. I/ml/h), in whom the mean PRA values were significantly higher ($p<0.005$) in the group with the most advanced retinopathy. LVH was present in one of the patients with Conn's syndrome; none of them had retinopathy grades II or III.

One of the patients with Conn's syndrome had also renal disease (pyelonephritis) with serum creatinine of 2.2 mg/100 ml. As she became normotensive after unilateral adrenalectomy, she had certainly not renal hypertension and is therefore not included in the renal disease group.

After exclusion of the patients with renal disease the remaining 54 patients were subdivided into a low PRA group of 10 patients (PRA ≤0.4 ng ang. I/ml/h, with no increase after ambulation and peroral furosemide) and a normal/high PRA group of 44 patients (PRA ≥0.5 ng ang. I/ml/h increasing in the upright position) (Table II). The low PRA group included the 3 patients with primary aldosteronism. Both the mean age, systolic and diastolic BP as well as known duration of hypertension and serum creatinine concentration were higher for the patients in the low renin group; only the difference in mean age was, however, statistically significant ($p<0.05$).

Table II shows the lower frequency of retinopathy grades II and III in the patients with low PRA occurring in 1 of 10 patients. In the patients with normal or high PRA, retinopathy grades II or III was found in 1 of 44. In contrast to this the relative frequency of LVH was even higher among the 10 patients with low PRA, in whom LVH was diagnosed in 5 as opposed to only 19 of the 44 patients with normal or high PRA.

Table III shows that the mean systolic BP in the low PRA group was significantly higher in patients with signs of LVH than in those without. This holds true also for the patients with normal or high PRA in whom the presence of LVH was associated not only with a higher systolic and diastolic BP but also with a significantly higher mean PRA than in patients without LVH.

Patients with LVH did not significantly differ in

Table II *Occurrence of LVH and the grade of retinopathy in 54 patients with arterial hypertension classif ed according to lo and normal/high PRA*

PRA group	*n*	Mean values for BP (mmHg) Syst	Diast	Age (y)	Known duration (y)	Serum creatinine (mg/100 ml)	PRA, mean and range (ng ang. I/ml/h) Supine	Upright	After furosemide
≤0 4	10	189	116	50.0	5.5	1 06	0.26 (0.2–0.4)	0.26 (0.–0.4)	0.3 (0.–0 4)
≥0 5	44	180	115	41 4	.83	0 97	1 (0 5–6 8)	3 2 (0 8–10 0)	
p		<0 30	<0 90	<0 05	<0.10	<0.30			

mean age known duration of hypertension or serum creatinine concentration from patients without LVH either in the low or the normal/high PRA group

In Table IV the same comparison is made for the grade of retinopathy In the high/normal PRA group retinopathy grades II–III was associated not only with a higher mean PRA but also with a higher mean systolic BP than retinopathy grade 0–I There was no significant difference in the mean age of the patients nor in the known duration of their hypertension but the serum reatinine concentration was significantly higher in those with the most advanced retinopathy In the low RA group only one patient had fundi changes ades II–III (grade II)

DISCUSSION

The finding of higher mean PRA in hypertensive patients w th renal disease than in patients with essential hypertension is in accordance with the observations of others (17) and so is the marked overlapping in individual values for PRA in the two groups a fact that makes the determination of PRA in peripheral venous blood of limited value in differentiation between essential and renal hypertension

For evaluation of the PRA the conditions under which it is determined are most important Brunner et al (6) have emphasized that the classification of essential hypertensives in low normal or high renin groups should be done only in relation to the patients sodium balance Whereas the influence of sodium restriction on the plasma concentration of renin and angiotensin II is a matter of discussion, present information indicates that PRA increases (3 5 8 10) Our own observations (unpublished) indicate that the adopting of an upright position in an individual on unrestricted sodium diet might be a more potent stimulus to renin release measured as PRA than sodium restriction in a recumbent individual

The PRA in the present study was determined after 48 hours sodium restriction with the patients recumbent after 10 hours in bed The stimulus of this regimen on PRA is probably of the same order or somewhat less than in ambulatory individuals on unrestricted sodium diet

As the PRA has not been higher than 0.4 ng ang I/ml/h in any of our patients with primary aldosteronism examined on this regimen we have somewhat arbitrarily chosen this PRA level as the upper limit for low suppressed renin An important criterion for inclusion in the low renin group has been that PRA should be not only low but also unresponsive The lack of increase in PRA after occupation of upright position and administration of furosemide clearly separated the low renin hypertension group from the other patients

Including 3 patients with Conn s syndrome and excluding patient with renal disease 10 of the patients (18 5%) had a low and non-responsi e PRA a somewhat lower figure than that reported in larger series of hypertensive patients selected according to other criteria (6 10) The effect on PRA of previous antihypertensive treatment was probably negligible as all medication of the treated patients was stopped at least 14 days before the study

We have found the mean PRA to be higher in

LVH (n)		Grade of retinopathy (n)	
Absent	Present	0–I	II–III
5	5	9	1
.5	19	3.	1

the patients with the most advanced retinopathy (grades II and III) and in patients with signs of LVH compared to patients with retinopathy grades 0–I and without LVH If PRA is assumed to re flect circulating angiotensin II concentration one may ask whether the positi e relation between a high PRA and hypertensive complications is an ex pression of a direct pathogenetic role of angiotensin for the development of LVH and retinopathy or whether they are all common manifestations of the increased arterial pressure I this connection the frequency of LVH and of retinopathy grades II and III in the low ren pa tients" compared to the frequency in the normal/ high PRA patients is of interest The occurrence of LVH in 50% of the patients with low PRA which wa even higher than in the normal/high PRA group contra ted with the paucity of changes in the optic fundi of these patients as opposed to the occurrence of retinopathy grades II and III in more than one fourth (28%) of the patient with normal/high PRA The difference in the distribution of the hypertens e complication in the two PRA groups could not be accounted for by dif ferences in mean BP serum creatinine concentration or in the known d ration of the hypertension

It is known that hyaline arteriolosclerosis may represent changes due to ageing and is ccentuated by chronic arterial hypertension (15) Age however could not be a factor of importance in the present patients since the mean age of the low PRA group n whom the retinopathy was less pronounced was significantly higher than that of the normal or high PRA group

From our observation it seems improbable that the development of LVH is d rectly influenced by the level of PRA In both PRA groups it was evident that the occurrence of LVH wa most closely related to the BP patients with LVH ha ing significantly higher BP than the patients without. Although the mean PRA wa also higher n the normal/high PRA patients w th LVH the findings strongly support the opinion of Pickering (4) that the le l of the BP is the more important factor for the de elopment of LVH that may be regarded as a response to increased working load

Although the number of patients small and the

Table III *LVH i rel tion to PRA and ome othe haracteri tic n 54 hyperten i patie t (mean value and ang)*

	PRA ≤0 4			PRA ≥0 5		
	LVH absent (n=5)	LVH present (n=5)	p	LVH absent (n=25)	LVH present (n=19)	p
PRA (ng ang I/ml/h)	0.3 (0.2–0 4)	0 26 (0._–0 4)	<0 99	1 65 (0 5–4 8)	2 68 (0 8–6 8)	<0 05
Systolic BP (mmHg)	177 (160–200)	200 (185–_10)	<0 05	172 (160–195)	19? (160– 30)	<0 001
Diastolic BP (mmHg)	113 (105–1_5)	118 (100–135)	<0 90	111 (95–130)	119 (160–_30)	<0 01
Age (y)	53 6 (39–67)	46 4 (38–64)	<0 90	40 0 (18–57)	43 3 (?7–58)	<0 30
Know duration of hypertension (y)	7.30 (0 5– 4)	3 70 (0 5–10)	<0 30	56 (0.5–15)	3 18 (0 5–11)	<0 60
Serum creatinine (mg/100 ml)	1 14 (0 8–)	1 0 (0 8–1 1)	<0 70	0 90 (0 6–1 2)	0 94 (0 7 1 6)	<0 90

Table IV *Retin opathy in relation t PRA and other haracteri tics in 54 hypert nsiv patients (mean value and range)*

	PRA ≤0 4			PRA ≥0.5		
	Grade of retinopathy			Grade of retinopathy		
	0–I (n=9)	II–III (n=1)	p	0–I (n=3.)	II–III (n=1)	p
PRA (ng ang I ml/h)	0 ?9 (0 –0 4)	0..		1.68 (0.5–4.8)	3..3 (0.8–6.8)	<0.005
Syst lic BP (mmHg)	186 (160– 10)	10		175 (160– 15)	193 (170–230)	<0.001
Dia tolic BP (mmHg)	115 (100–135)	1?0		111 (95–130)	1..3 (105–135)	<0 001
Age (y)	48 4 (38–67)	64 0		40 0 (18–57)	45.. (?7 58)	<0 30
Known d ration of hyper tension (y)	5 55 (0 5– 4)	5 0		..69 (0 5–15)	3...1 (0.5–10)	<0 70
Serum creatinine (mg/100 ml)	1 1 (0.8–)	0.8		0.89 (0.6–1)	1.01 (0.7 1.6)	<0.05

limitations of fundoscopy being a subjective method should not be forgotten the finding of less severe arteriolar changes n the fundi of patients with low suppressed PRA compared to the more serious changes in the patients with normal or high PRA could support a pathogenetic role of angiotensin *for the development of arteriolar damage* especially since there was no difference in mean ur in renal function judged from the serum tinine concentration in the two groups Nei was there any statistically significant dif ence n the known mean duration of the hypertension but thi of ourse i an uncertain measure of the real duration

The finding in the normal/high PRA group that *the higher mean PRA in the patients with the most* accentuated arteriolar changes was also accompanied by a higher mean arterial BP as well as by a higher mean serum creatinine concentration does not exclude an independent or at least a synergistic role of angiotensin for the development of hypertensive arteriolopathy Irrespective of the primary cause in some patients a vicious ircle might be started in which a high rterial BP renal arteriolar damage and increased secretion of renin are both cause and result

The idea that renin or rather angiotensin may cause vascular lesions is not a new one Clinical observations (3 4 6 10 17 ?0) have shown a positive association between retinal arteriolar changes and the concentration of plasma renin and angiotensin as well as PRA Moreover arteriolar damage has been induced in animals after administration of angiotensin or renin-containing renal extracts (11 15) This effect however has not been observed without a concomitant rise in BP The vasculotoxic effect of renal extracts has *been shown to be less evident after bilateral* ad enalectomy (11) an indication that aldosterone might also be implicated in the pathogenesis presumably by its effect on sodium balance The role of sodium is supported by experiments on rats in which vascular disease was elicited by long-term injections of renin (?1) The pathological changes were accentuated when the animals were pretreated with aldosterone and sodium but not *when sodium was omitted from the diet*

The etinal arteriolar changes are representative of similar changes in other tissues (15) This pathological process is however quite different from the process of arterial atherosclerosis the development of which is accentuated by rterial hypertension (16) the most probable explanation of the increased ncidence of cerebral thrombosis and coronary infarction in patients with hypertensive disease (19) We do not know any experimental observations that might support the hypothesis that renin–angiotensin directly promotes the development of atherosclerosis There would therefore be no reason to expect as reported by Brunner et al (6) that the incidence of coronary inf rction and cerebral thrombosis would be lower

in patients with low PRA than in other patients with comparable degree of hypertension. In other studies (1 22 26) the observations of Brunner et al ha e not been confirmed Both for this reason and from the fact that the development of LVH seems relatively independent of the level of PRA it would not be wise as suggested by Laragh s group (6 20) to delay the treatment of high BP in patients w th low PRA Laragh s (20) suggestion that antihypertensives which also reduce PRA, ought to be preferred to agents which stimulate renin release seems more justifiable since the available data suggest that a high plasma level of angiotensin may accentuate hypertensive arteriolar damage

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CHANGES IN HEPATIC CIRCULATION AT REST DURING AND AFTER EXERCISE IN YOUNG MALES WITH INFECTIOUS HEPATITIS COMPARED WITH CONTROLS

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Abstract Hepatic venous catheterization has been performed in 10 young males with infectious hepatitis and 13 healthy male volunteers. Hepatic blood flow at rest in the supine position, estimated with indocyanine green dye in constant infusion, was significantly higher in the patients than in the control (2.1 and 1.44 l/min respectively). During supine leg exercise with heart rates about 170 beats/min the absolute reduction of total hepatic blood flow was similar in patients and control. The arterio-hepatic venous oxygen difference during exercise as compared with that at rest was doubled in the patients, but tripled in the control. The splanchnic oxygen uptake at rest was significantly higher in the patients than in the controls and did not change significantly during exercise. At rest the patients had significantly greater difference between wedged and free hepatic venous pressure than the controls possibly due to swelling of the hepatocytes. The hyperkinetic hepatic circulation might have been caused by cellular damage and blood flow impairment with anoxia and metabolically evoked vasodilatation and also by an increased regeneration of liver cells, as splanchnic oxygen uptake was elevated. Immunologic factors may also have contributed. No negative effects of the exercise were noted in 8 patients with serum bilirubin values below 4 mg/100 ml, whereas relapse and slightly delayed recovery were noted in patients with serum bilirubin values of 9.6 and 5.8 mg/100 ml, respectively.

Only a limited number of studies on the hepatic circulation in patients with acute viral hepatitis have been published so far and measurements have been limited to the resting state (6, 9, 19, 1, 23, 4, 27). The hepatic extraction of colloid particles such as radioactive gold or chromic phosphate was found to be normal (27) or decreased (6, 19) interpreted as a normal or decreased hepatic blood flow. By intrasplenic injections of radioactive iodinated serum albumin and external or blood counting in 8 patients it was concluded that their hepatic blood flow was normal (4). Similarly in 14 patients Preisig et al. (1) calculated normal values for hepatic blood flow using indocyanine green (ICG) dye as indicator.

Different methods have been used to find out whether or not the portal venous pressure is increased in these patients. Preisig et al (1) found normal values for wedged hepatic venous pressure as a measure of the hepatic sinusoidal pressure. On the other hand, increased intrasplenic pressures have been observed in about half of the patients studied (3). Even esophageal varices have been observed esophagoscopically but not radiologically in 35 of 87 patients (9). Thus, previous studies in patients with viral hepatitis at rest have shown fairly normal hepatic blood flows and signs of normal or slightly increased portal venous pressures.

When a normal subject starts to exercise the increase in activity of the sympathetic adrenergic fibers reduces the hepatic blood flow like the flow to most non-exercising parts of the body (2, 26, 33) and similarly a reduction occurs when changing from supine to standing position (3). Despite the absence of similar studies in patients with viral hepatitis they have since long been treated with bed rest during the acute phase of the disease. The bed rest has varied from very strict (1) to moderate (7, 3) and has been used to prevent a reduction of hepatic blood flow and thereby a possible delay of healing. However strenuous exercise for 3 hours daily did not affect recovery time or relapse rate as compared with the patients

treated conventionally (25) Similar results were observed in patients with viral hepatitis who per formed a moderately heavy bicycle exercise (8).

The present investigation a preliminary report of which has already been published (17) was therefore undertaken in patients with infectious hepatitis to find out whether or not their hepatic circulation was normal at rest whether the flow was reduced in the normal way during supine leg exercise and whether this exercise caused any signs of impaired liver function

MATERIAL

After their informed consent 10 afebrile males with HBAg-negativ viral hepatitis and 13 healthy male olunteers were studied in the morning without pre medication. Although being sporadic the cases were diagnosed as infectious hepatitis since clinical and laboratory findings were not consistent ith infection due to Epstein-Barr irus or to cytomegalovirus, the onset of the disease was acute and the patients were HBAg-negative as early as 5 (2-8) days after onset of jaundice. None of the 23 ubjects all 20-30 years old had a previous history of heart, lung, kidney or li er disease or of drug abuse and none used alcohol in exces In each group 3 ubjects were well trained, and the others normally trained except patient 10 who was untrained. Prior to the study all patient had been allowed ambulance in the ward The first patients were investigated just prior to their discharge. As no negativ of the exercise were observed the relativ work was increased and subjects in an earlier phase of disease were studied On the day of the study 8 pa ient had serum bilirubin values below 4 mg/100 ml ($\bar{x}$=1 99) SGOT below 150 U ($\bar{x}$=70 3) and SGPT below 400 U ($\bar{x}$=224 4), whereas the corresponding average values in the other patients were 7 7 mg/100 ml, and 515 and 855 U (Table I) The first 6 patients and 9 controls were allowed a light breakfast 3 hours be fore the flow study The remaining 4 patients and con trols were studied after overnight fast

METHODS

The subjects were tudied during a resting period of about 1 hour followed by two consecutive 20 min periods of supine leg exercise on an electrodynamically braked bicycle ergometer (10) and during 30 min after exercise. The second work load was generally twice as high as the first and chosen so as to correspond to the heaviest load the subject was expected to sustain for another 20 min period. In 7 patients and 4 controls a 15 min rest was inserted between the loads to study the wedged and free hepatic enous pressures also after the first load. Most of these subjects also parti cipated in another study (18) to estimate the hepatic fat content by retrograde injections of solutions of ^{85}Kr and ^{133}Xe in saline into an occluded hepatic vein be fore the exercise No systematic influence of these in jections on the hepatic extractions of oxygen and ICG were observed when measured both before and 2-4 min after the injections in patients and controls

Catheterization W th the subject in the supine posi tion a right hepatic vein was percutaneously catheter ized from cubital vein under TV fluoroscopy and the radiopaque teflon cathete (1../2.0 or 1.5/2.3 mm) was localized as far caudally and peripherally as pos sible In the wedged position the tip of the catheter w s generally located -4 cm from the lateral chest all and in the free position about 4 cm more proximal. Short teflon catheters (1 1/1 4 mm) were also percu taneously inserted into the brachial artery and a periph eral en In patient and 5 controls another right hepatic vein wa similarly catheterized from the contra lateral arm In 3 patients and 4 controls a thin poly ethylene catheter (PE 60 0.76/1.22 mm) was also per cutaneously inserted from cubital vein into the pul monary artery

Blood-flow measurement After a primary dose of about 15 mg ICG (Cardio-Green® Hynson Westcott and Dunning, Baltimore Md. USA) a constant i. in fusion of the dye was gi en at a rate of about 1 mg/ min. After an equilibration period of bout 30 min 3-5 paired blood samples from the artery and hepatic ein wer collected, the arterial sample about 15 sec prior to the enous one Blood was draw at generally 5-min interval at rest during the tw work loads and the pause between them if any and after exercise (Fig 1) The plasma content of ICG was determined on a H tachi 101 spectrophotometer (Hitachi Ltd Tokyo Ja pan) after correction for plasma turbidity at 900 nm (20) Hematocrit was determined on all arterial samples by spinning the blood for 5 min at 9 000 g in a micro hematocrit centrifuge (MB International Equipment Co, Needham, Mass. USA). Plasma and blood olumes were estimated at rest and 30 min after exercise by in jection of about 3 µC ^{131}I-human serum albumin. Total hepatic blood flow at rest was calculated by the Bradley technique according to Winkler et al. (36) from the average arterio-hepatic enous dye difference and the eventual average change in arterial ICG concentra tion. During and after exercise corrections for ariation in arterial dy concentration due to changes in plasma volume were performed mainly according to Hultman and Casterfors (11) The hepatic extraction of the dye was measured a the arterio-hepatic venous difference divided by the arterial concentration

Oxygen saturation and Hb concentration were deter mined on a CO oximeter (IL 182, Lexington, Mass., USA) calibrated against cyanmethemoglobin Splanch nic oxygen uptake wa calculated as total hepatic blood flow×arterio-hepatic venous oxygen difference Heart rate was determined from ECG recorded on a UV writer (see below) In 3 patients and 4 controls total oxygen uptake was determined by the Douglas bag technique and micro Scholander analyses and cardiac output and stroke volume wer calculated according to the Fick principle

Table I *Anthropometric clinical and laboratory data on the day of the study and their maximal abnormalities prior to the study in 10 patients and corresponding group mean values for 13 controls*

BSA = body surface area, SBil = serum bilirubin ALP = alkaline phosphatase Thy = thymol turbidity RCM = right costal margin

	Age (y)	Height (cm)	Weight (kg)	BSA (m^2)	Work load (kpm/min) I	Work load (kpm/min) II	Days after onset of jaundice	SBil (mg/100 ml) <1.3	*ALP* (U) <3.0	*LDH* (U) <225	*SGOT* (U) <40	*SGPT* (U) <35	Thy (U) <6	Liver size palpable below RCM (cm)
							On the day of catheterization							
Pat. no.														
1	29	177	67	1.85	300	600	15	1.6	6	152	43	146	9	1
2	26	181	73	1.95	350	700	23	1.8	2.4	184	81	206	11	0
3	27	182	67	1.89	250	500	14	5.8	4.0	199	320	970	6	1.5
4	25	186	85	2.11	400	800	12	1.8	2.6	145	170	213	2	0
5	21	180	62	1.82	400	700	14	1.4	.5	126	36	150	2	0
6	22	186	72	1.98	600	1 000	10	9.6	2.7	339	710	740	13	2
7	25	186	80	2.06	500	1 000	22	3.5	.9	132	79	350	4	2.5
8	23	185	75	2.00	350	700	15	1.1	3.5	170	78	360	14	
9	25	190	83	2.13	350	700	17	2.6	2.5	134	37	100	1	1
10	27	169	63	1.73	300	500	16	2.1	2.6	141	88	270	–	
$\bar{x}$	25.0	182.2	72.7	1.952	380.0	720.0	15.8	3.13	2.83	172.[illegible]	199	350.5	8.1	1
S.D.	2.4	6.0	8.1	0.130	103.3	175.1	4.0	2.66	0.57	63.3	210.4	284.1	4.7	0.9
Controls														
$\bar{x}$	24.8	182.2	74.5	1.973	496.2	984.6	–	0.87	1.84	159.7	18.5	16.9	1.4	
S.D.	2.3	5.3	6.7	0.109	85.3	167.6		0.25	0.34	32.4	4.8	6.3	0.7	
Diff.	0.2	0.0	−1.8	−0.021	−116.2	−264.6	–	2.26	0.99	12.5	140.7	333.6	6.7	
p	>0.80	>0.99	>0.50	>0.60				**	**	>0.50	***	**		
							Maximal abnormalities prior to catheterization							
1							6	8.5	4.8	254	310	1 000	17	2
2							6	11.7	3.8	237	202	1 000	.3	1
3							6	11.9	5.2	466	1 000	000	12	3
4							6	3.3	4.3	47	780	570	2	0
5							5	5.4	4.9	411	880	1 000	6	1
6							6	12.2	3.1	777	780	1 000	23	3
7							5	14.4	7.5	546	1 000	1 000	20	4
8							2	4.3	4.7	579	1 000	2 000	11	3
9							8	16.6	3.8	290	950	1 000	15	2
10							2	10.7	5.2	499	1 300	2 700		3
$\bar{x}$							5.2	9.90	4.73	380.6	820.2	1 377.0	14.3	
S.D.							1.9	4.42	1.19	134.3	337.2	667.1	7.3	1.[illegible]

$^*p<0.05$ $^{**}p<0.01$ $^{***}p<0.001$ where p is the probability that the difference between the groups was caused by random factors. Upper normal limits given below each biochemical liver test.

Wilcoxon's nonparametric testing (28).

Total blood loss did not exceed 250 ml, except in the last 4 patients and controls, in whom 100 ml extra blood was drawn for metabolic studies

Intravascular pressures were measured by transducers (Bell & Howell no. L 221 Woking, Surrey England) using an EMMA amplifier (SE labs, Feltenham Middlesex, England) and UV writer (SE 3006, SE labs). Reference point for zero pressure was the mid-thoracic level at the insertion of the fourth rib on the sternum. Free and wedged hepatic venous pressures were repeatedly recorded at rest during quiet breathing with large amplification T check whether or not properly wedged catheter position was obtained, the undamped and damped curve was analysed For each measurement the pressure of the damped curve was integrated manually over 20-30 sec and tated to the nearest 1/10 mm Hg (16)

Prior to blood sampling the arterial BP was similarly recorded Splanchnic flow resistance was derived as the fall in pressure (i. mean arterial BP minus free hepatic venous pressure) divided by total hepatic blood flow Postsinusoidal flow resistance was derived as the fall in pressure from wedged to free hepatic vein divided by total hepatic blood flow

Biochemical liver tests Serum bilirubin SGOT SGPT LDH alkaline phosphatase and thymol turbidity

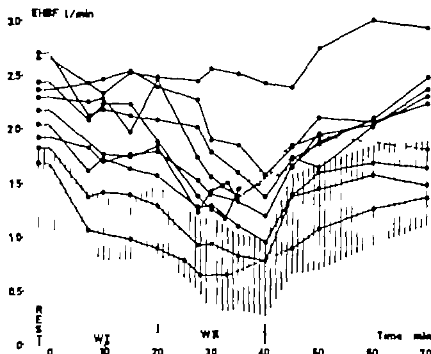


Fig 1 Estimated hepatic blood flow (*EHBF*) at rest (O) during two supine bicycle work loads (*WI* and *WII*), and after exercise (●) In the patient -- discontinued work load, hatched area = range of the controls.

were determined by standard methods on blood sampled at rest before the flow study These tests were also performed on blood sampled 30 min after exercise on the 3rd or 4th and on the 6th day after catheterization and then once a week until the patient was discharged and generally at 3–4 week intervals until their normalization

Statistical calculations were made by standard methods (28). The following probability levels of significance were used: $p<0.001$ highly significant, $p<0.01$ significant and $p<0.05$ probably significant.

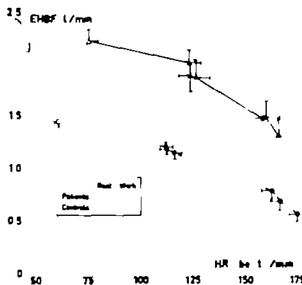


Fig 2 Estimated hepatic blood flow (*EHBF*) at rest (unfilled symbols) after 10 and 15 min and at the end of two generally 20 min periods of supine leg exercise (filled symbols) in relation to heart rate (*HR*) in the patients (Δ—▲) and the controls (O—●) Group means±S.E.M. No. of individuals is given when reduced

RESULTS

The patients were on an average studied at a heaviest work load of 720 kpm/min and a heart rate of 166 beats/min, as compared with 985 kpm/min and 175 beats/min for the controls. The difference in work load was significant but not the difference in heart rate In relation to work load the patients had a heart rate about 15 beats/min higher than had the controls Total blood volume was equal in patients and controls on an average 5 27 and 5 24 l respectively Due to exhaustion 4 patients and 7 controls had to terminate the second work load after 10–19 min, and on an average this work load lasted for 18 75 and 17 60 min for patients and controls respectively For both groups final values are given for this work load The change in heart rate during the heaviest load was the same in both groups, 8–14 beats/min from the 10th min to the final value (Fig. 2).

Table II *Some circulatory data at rest (RI) at the end of th first (WI) and the second ork load (WII) and 30 min afte exercise (RII) in the patients and controls*

	Estimated hepatic blood flow (l/min)				Arterio-hepatic venous xygen difference (ml/l)				Splanchnic oxygen uptake (ml/min)			
	RI	WI	WII	RII	RI	WI	WII	RII	RI	WI	WII	RII
Patients												
$\bar{X}$	[illegible]1	1.87	1.30	2.07	36.1	45.7	74.9	42.9	79.7	82.6	89.8	87.4
S.D.	0.34	0.57	0.51	0.57	4.8	8.8	25.3	8.0	16.0	70.	28.9	1.4
	10	10	10	9	10	10	10	9	10	10	10	9
Controls												
$\bar{X}$	1.44	1.15	0.56	1.48	43.0	56.9	130.9	46.4	60.9	65.4	67.1	66.8
S.D.	0.21	0.22	0.21	0.[illegible]3	7.8	10.9	34.7	8.7	9.[illegible]	15.9	12.9	12.9
	13	13	1	12	12	1	11	1	1	1	11	11
Difference	0.77	0.77	0.74	0.59	−6.9	−11.2	−56.0	−3.5	18.8	17.2	[illegible]7	20.6
p	**		***				**	>0.30				

	Heart rate (beats/min)				Mean arterial BP (mmHg)				Splanchnic flow resistance (mmHg/l/min)			
	RI	WI	WII	RII	RI	WI	WII	RII	RI	WI	WII	RII
Patients												
$\bar{X}$	75.2	125.9	165.6	96.5	88.3	92.2	98.6	84.8	37.5	50.8	87.6	40.9
S.D.	14.0	19.9	21.8	14.6	10.0	6.7	3.9	10.5	8.9	[illegible]3.6	41.3	14.6
	10	10	10	10	10	10	10	10	10	10	10	9
Controls												
$\bar{X}$	60.6	116.1	175.0	82.8	90.7	100.8	110.	90.5	60.0	83.8	204.5	57.0
S.D.	7.9	13.1	14.6	10.8	9.6	4.9	7.4	8.6	9.7	17.5	94.9	10
	13	13	13	13	12	13	12	11	1	13	11	9
Difference	14.6	9.8	−9.4	13.7	−2.4	8.6	−11.6	−5.7	−22.5	−33.0	−1 1.9	−16.1
p	**	>0.10	>0.20		>0.40		**	>0.10			**	

$p<0.05$ ** $p<0.01$ ** $p<0.001$ where p is the probability that the difference between the groups was caused by random factors

Hepatic blood flow The hepatic circulation at rest in the patients was characterized by larger total hepatic blood flow lower splanchnic flow resistance lower arterio-hepatic venous oxygen difference and higher splanchnic oxygen uptake than in the controls (Table II). Neither in patients nor in controls was total hepatic blood flow related to any of the body-size variables. At the highest work load the hepatic blood flow of the patients had decreased to an average of 59% of the value at rest as against 39% in the controls. The hepatic blood flow in relation to heart rate as a measure of the relative work load is given for both groups in Fig. 2. When the reduced hepatic blood flow during exercise was expressed in % of the value at rest the patients and the controls had the same flow reduction in relation to the increase in heart rate (Fig. 3). Although the mean arterial BPs during exercise were significantly

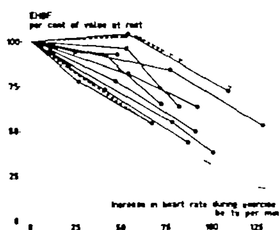


Fig 3 Change in estimated hepatic blood flow (*EHBF*) during exercise (end of two generally 20 min periods of supine leg exercise) relation to change in heart rate in the patients. The area inside the broken line=range of the controls.

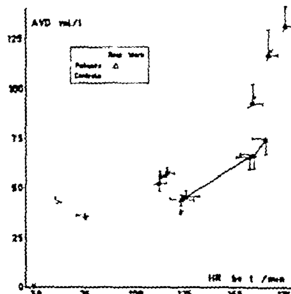


Fig 4 Arterio-hepatic venous oxygen difference (AVD) at rest (unfilled symbols) after 10 and 15 min and at the end of two generally 20 min periods of supine leg exercise (filled symbols) in relation to heart rate (HR) in the patients and controls Group means±S E.M No of individuals given when reduced Symbols as in Fig ...

lower in the patients than in the controls (Table II) the increases in splanchnic flow resistance with exercise were not significantly different when related to the simultaneous increases in heart rate

At 30 min after exercise all variables except heart rate returned to the initial values at rest in both groups the heart rates being elevated about 70 beats/min ($p<0.001$) (Table II)

Hepatic extractions In the patients the average hepatic venous oxygen saturation was reduced from 79% at rest to 74 and 60% respectively at the end of the first and second work loads The corresponding values for the controls were 76% at rest and 70 and 36% during exercise The resultant lower arterio-hepatic venous oxygen difference at rest in the patients as compared with the controls was still more marked during exercise (Table II Fig. 4) At 30 min after exercise the hepatic venous oxygen saturation and the arterio-hepatic venous oxygen difference had not completely returned to the resting values in the patients ($p<0.01$) in contrast to the controls ($p>0.10$) The average splanchnic oxygen uptake at rest was significantly higher in the patients than in the controls (Table II) In neither group was the splanchnic oxygen uptake during or after exercise significantly changed from the resting level, indicating that the increase in arterio-hepatic venous oxygen difference during exercise compensated the decrease in blood flow Splanchnic oxygen uptake at rest as a fraction of total oxygen uptake was on an average 0 34 (range 0 30–0.37) in 3 patients and 0 25 (range 0 20–0.29) in 4 controls

The hepatic extraction of ICG at rest was 54 (range 27–75)% in the patients and 75 (range 65–89)% in the controls the difference between the groups being significant. Among the patients those with high serum bilirubin values had the lowest extraction ($r=-0.856$).

Hepatic venous pressure The average wedged hepatic venous pressure at rest in the patients was not significantly different from that in the controls (Table III) Also the free hepatic venous pressure was similar in patients and controls both before and after exercise In both groups wedged and especially free hepatic venous pressure decreased during exercise During the 15-min pause between the work loads the average wedged hepatic venous pressure was unchanged as compared with that before exercise for both patients and controls The average difference between wedged and free hepatic venous pressure was significantly higher at rest in the patients as compared with the controls (Table III) In both groups the pressure difference increased after exercise but this change was significant for the controls alone ($p<0.01$) After the first work load the pressure difference was 3 4 mmHg in the patients and 1 1 mmHg in the controls.

Postsinusoidal flow resistance at rest was significantly different between patients and controls on an average 1 30 and 0.69 mmHg/l/min respectively

Relationships between hepatic circulation and laboratory and clinical data were studied In the patients total hepatic blood flow arterio-hepatic venous oxygen difference duration after onset of jaundice and liver size were not significantly related to any of the variables. Relationships of significance are given in Table IV In the controls the only correlation of any significance was between the serum bilirubin and the difference between wedged and free hepatic venous pressure ($r=0.645$)

Effect of exercise on symptoms and biochemical liver tests In the 8 patients who at the time of

Table III *Wedged hepatic venous pressure (WHVP) and the difference between wedged and free hepatic enous pressure (W FHVP) at rest (RI) and after the second work load (RII) in 9 patients and 11 controls*

	WHVP (mmHg)			W-FHVP (mmHg)			
	RI	RII	RII-RI	RI	RII	RII-RI	RII/RI
Patients							
$\bar{x}$	10.1	9	−0 9	_9	3 4	0.6	1 2
S.D.	2.2	1.8	0 9	1 1	1 6	0 9	0 4
Controls							
$\bar{x}$	8.7	7 0	−1 7	0.9	1 9	1.0	2.8
S.D	1 7	1.8	1_	0.5	0.7	0 9	_6
Difference	1 4	_2	0 8	_0	1.5	−0 4	−1.6
p	>0.10		>0.10	**		>0.30	>0 05

$p<0.05$ ** $p<0.01$ ** $p<0.001$ where p is the probability that the difference between the groups was caused by random factors.

the study had serum bilirubin below 4 mg/100 ml and SGOT and SGPT below 150 and 400 U (Table I) no negative effects of the exercise were noted on recovery or relapse rate Of the other patients no 3 showed a normal recovery after exercise and the alues of his liver tests were lower on the 4th day than before catheterization On the 6th day without aggravated symptoms a slight and temporary increase was noted for SGOT from 300 to 390 U whereas SGPT and serum bilirubin were unchanged. Thereafter all the liver test values decreased and the recovery was uneventful. In patient 6 serum bilirubin decreased to 8 4 mg/100 ml on the 4th day but SGOT and SGPT both rose to 1000 U On the 6th day the corresponding values were 7 1 960 and 1000 On the 10th day the patient experienced malaise fatigue loss of appetite and increased jaundice On the 11th day serum bilirubin had increased to 11.3 mg/100 ml whereas SGOT and SGPT had decreased to 700 and 840 U respectively One week later he began to improve clinically in spite of a serum bilirubin value of 13 1 mg/100 ml SGOT and SGPT were down to 460 and 490 U respectively Thereafter serum bilirubin decreased and the recovery was uneventful

Table IV *Relationships between hepatic circulatory and laboratory data in the patients and the total material*

Correlation coefficient is only given when at least probable significance was observed
SBil=serum bilirubin, W FHVP=difference between wedged and free hepatic venous pressure

	Splanchnic o ygen uptake	W-FHVP	Total hepatic blood flow
Patients			
SBil	0 814*	0.890*	n.s
SGOT	0 838*	0.883	s.
SGPT	0.853**	0 666	n s.
W-FHVP	0 761		
Total material			
SBil	0 793**	0.866 **	0 627*
SGOT	0.783	0.805	0.571**
SGPT	0 835	0.803	0.749*
W FHVP	0 717*		0 669*

$p<0.05$ ** $p<0.01$ ** $p<0.001$ .s =not significant.

DISCUSSION

As the data of the controls were in good agreement with results obtained by others (3 26 33 34 36) both at rest and during exercise the changes in the patients were evidently related to the disease This is reinforced by the observation of normal hepatic circulation at reexamination of 3 patients 5 weeks after the present study (15).

The patients were studied on an average at lower work loads than the controls, mostly due to lower physical working capacity However the pertinent comparisons during exercise between patients and controls were made in relation to heart rate as a measure of the relative work load since the decrease in hepatic blood flow during exercise has been found to be more closely related to rela-

tive than to absolute work load (26) Thus the present differences in work load between patients and controls are of minor importance At re examination about 5 weeks later of 3 of the pres ent patients (15) their heart rates at rest and during exercise were on an average 13–20 beats/min lower than during the first study Therefore the higher heart rates during exercise of the patients as compared with the controls seem to have been caused by the disease or its treatment with bed rest and not by selection or differences in pre morbid physical capacity The experimental conditions varied slightly as in 7 of 10 patients and 4 of 13 controls a 15-min rest was inserted between the two work loads In 6 of the patients and 9 of the controls a light breakfast was also allowed 2–3 hours prior to the flow study whereas the others were studied after overnight fast By group comparison however no effects of these variations of any significance were observed on the variables studied or their variation with exercise

Blood is generally sampled from only one hepatic vein on the assumption that this sample is representative of all hepatic venous blood but variations in dye extraction have been observed with catheters positioned in different right hepatic veins (4) In one patient with moderate liver cirrhosis and 7 subjects without liver disease (36) significant differences in extraction between the two usually rightsided hepatic venous catheters seen in about 5% of the determinations but ...ally only for one or two of the 5 substances Great simultaneous regional differences in extraction of ICG and oxygen have been noted in alcoholics with marked cirrhosis of the liver (30) indicating heterogeneity of metabolic activity in relation to blood flow perhaps related to regional variations in cell regeneration (31) In the present study tests for the occurrence of gross heterogeneity of hepatic circulation were made in 2 patients and 5 controls by simultaneous sampling from an additional catheter located in a more cranial right hepatic vein both at rest during and after exercise However there was no difference of any significance between the two hepatic veins with respect to ICG concentration or oxygen saturation. Thus hepatic blood flow and oxygen extraction seem to be similar in different parts of the right liver lobe at least in areas of this size which is in agreement with the finding of no gross heterogeneity in the distribution of ^{198}Au in photoscans of 8 patients with viral hepatitis (21)

The hyperkinetic hepatic circulation was not part of a general vasodilatation. In 3 patients and 4 controls cardiac output was determined simultaneously with hepatic blood flow The patients had a mean cardiac output at rest of 8 7 l/min and a mean hepatic blood flow of 2.6 l/min, or 30% of the cardiac output. The corresponding values for the controls were 7 3 and 1.5 l/min and 20% respectively The average flow of blood to extrahepatic parts of the body was thus the same in both groups

The high hepatic blood flows in acute infectious hepatitis agree with the hyperemia in the diseased organ in many other infections However in previous studies (6 19 21 24 27) no flow increase was observed although in one of them (21) the reliable method with constant infusion of ICG was used. In earlier studies only the hepatic mesenchymal clearance of radioactive gold (6 19) and chromic phosphate (27) was measured which is not necessarily related to hepatic blood flow

It has been shown in man that total hepatic blood flow could increase markedly after typhoid vaccination even when fever was prevented (3). In rabbits an increased clearance of ICG was observed for many weeks after i v injections of heterologous protein (14) which was considered to be due to an induced stimulation of the parenchymal cells may be by a higher blood flow Our present data do not allow any conclusions as to whether or not immunological factors contribute to the observed high hepatic blood flows in acute infectious hepatitis

A more probable cause of the high hepatic blood flow might be the hepatic cellular damage and regeneration unevenly distributed within the lobules A local vasodilatation evoked by such changes can only be mediated by the hepatic artery as a decrease in the small portal venous flow resistance over the liver is unlikely and anyhow cannot measurably increase the portal venous blood flow On the contrary an increased flow resistance over the portal sinusoids should be expected as in fatty liver and toxic hepatitis (22), where the swollen hepatocytes compress the sinusoids, increase the sinusoidal flow resistance and impair the local blood flow If the hepatic blood flow in infectious hepatitis is similarly unevenly impaired within the lobules anoxia will develop

in some areas A resultant metabolic vasodilatation will correspond to the most anoxic regions rather than to the average need and the flow increase in excess of the metabolic demands will result in a lower arterio-hepatic venous oxygen difference and a higher oxygen saturation in the hepatic venous blood as was found in the present study

The increased difference between wedged and free hepatic venous pressure in the present patients as compared with the controls was explained by the increased postsinusoidal flow resistance possibly due to cellular swelling, but altered microcirculation with increased hepatic artery flow might also have contributed. Although the absolute value of the difference between patients and controls was low it corresponded to a –3 times higher flow resistance Despite these significant changes the wedged hepatic venous pressures were not significantly higher in the patients than in the controls which findings are in good agreement with those of Preisig et al (21). The signs of increased portal venous pressures in viral hepatitis reported in other materials (9 13) probably only indicate somewhat more marked changes than in the present study

There were significant correlations between the difference between wedged and free hepatic venous pressure and the state of the disease as estimated by biochemical liver tests even if only small absolute differences were noted between patients and controls before and after exercise

The indications of larger than normal oxygen consumption during regeneration of hepatic tissue (5) might explain the present observation that both splanchnic oxygen uptake and blood flow were increased In our patients the biochemical liver tests were more closely correlated to the splanchnic oxygen uptake than to the total hepatic blood flow that is more influenced by other factors e.g. the sympathetic activity at the time of the study

In the last 4 patients and controls blood samples were analysed for studies of carbohydrate protein and fat metabolism (Lundbergh Furst Strandell and Wahren personal communication 1974), but so far no major abnormalities have been observed. The high splanchnic oxygen uptake of the patients could have been caused by an increased hepatic combustion of fat which increases the splanchnic oxygen uptake compared with the situation when predominant carbohydrate metabolism occurs (12) However in the present material there was no significant difference in respiratory quotient between 3 patients and 4 controls the average value being 0 87 (range 0.84–0.94) for the patients and 0 77 (range 0 70–0 92) for the controls The dietary and medical treatment given the patients cannot have affected the hepatic circulation significantly: only once or twice a week was the normal hospital food replaced by a meal less rich in fat and the only medical treatment given was vitamins orally twice the daily need

The flow increase can have been related to cellular changes and to immunological responses In both instances the hepatic artery blood flow should be increased but an immunological response should also include an increased splenic blood flow No data on the distribution of splanchnic blood flow in viral hepatitis are yet at hand but methods to separate these flows in awake patients are now available (29) They necessitate the insertion of multiple portal venous catheters (35) and have not been used so far Indirect evidence of an increased hepatic artery blood flow was however present in our patients since in one case a wedged hepatic venous pressure was recorded after exercise with one marked wave resembling an arterial curve and a pressure level close to the arterial one (17). In others unstable wedged pressure recordings were seen after exercise with sliding pressures up to 30–40 mmHg which cannot represent portal venous pressure.

In spite of great differences in flow values the decrease in hepatic blood flow during exercise was equal in both groups in relation to the increase in heart rate indicating the same sympathetic flow control in both groups

During and after the second world war epidemics of infectious hepatitis occurred in many countries and some of the patients treated in military hospitals constituted materials for studies regarding therapy The importance of strict bed rest and high protein intake in viral hepatitis was stressed (1). This opinion was unchallenged until other authors (7 34) reported that strict bed rest was not superior to bed rest ad libitum after the acute phase Deleterious effects of vigorous exercise during the first days of the preicteric phase have been observed in one study (13) 3 of 5 patients died in hepatic coma within 4–16 days after onset of disease but no figures were reported

of how often physical exercise was performed during the preicteric stage To establish when heavy exercise may be allowed without risk in viral hepatitis patients have been submitted to exercise In Vietnam 398 American servicemen with infectious hepatitis were included in such a study (25) Half of them were ambulant in the ward without prescribed exercise whereas the other half participated in daily exercise for 3 hours as soon as their symptoms were considered slight average serum bilirubin was 7.5 mg/100 ml. No adverse effects of the exercise were noted on recovery or relapse rate However neither the material nor the intensity of the work was clearly defined *These findings were reinforced by a study in 23* patients with acute viral hepatitis who performed 1 min of moderately heavy bicycle exercise for 6 consecutive days (8)

In the present material none of the 8 patients with values of serum bilirubin below 4 mg/100 ml SGOT and SGPT below 150 and 400 U at the time of the catheterization showed any signs or symptoms of negative effects of the exercise Of the patients with serum bilirubin values of 5.8 and 9 6 mg/100 ml respectively no 3 showed a slight delay in the decrease of the liver-test values and no 6 a relapse that delayed his recovery for about weeks. Patient 3 performed only 500 kpm/min with a final heart rate of 170 beats/min, whereas patient 6 was well trained and performed a heaviest load of 1000 kpm/min with a final heart rate of 180 beats/min These prolonged recoveries may have been due to chance as this clinical course occurs in about 10% of patients with acute viral hepatitis according to current text books and the experience in this hospital However the possibility cannot be excluded that the physical exercise may have been the provoking factor and pending more extensive studies heavy physical exercise should be avoided until after the acute phase e g. when serum bilirubin is down to about 4 mg/100 ml

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Lp(a) LIPOPROTEIN AND PRE β_1 LIPOPROTEIN IN YOUNG ADULTS

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Abstract A ery strong association between phenotype Lp(a+) and presence of pre-β_1-lipoprotein has been observed in tw series of healthy young adults. The data are in agreement with the iew that pre-β_1-lipoprotein is identical to the lipoprotein carrying the Lp(a) antigen the Lp( ) lipoprotein. Lp(a) antigen/pre-β_1-lipoprotein occurred more frequently in persons with positive than th negative family history of coronary heart disease though this trend was not statistically significant Occurrence of Lp( ) antigen/pre-β_1-lipoprotein was not correlated ith smoking or with the use of contraceptiv pill

The Lp(a) antigen is a genetically determined trait of human serum lipoprotein (1) The antigen as defined by standardized techniques and antisera in our laboratory is present in about 35% of healthy people of Western European extraction and it resides in a distinct species of lipoprotein molecules, the Lp(a) lipoprotein. This lipoprotein migrates faster than β-lipoprotein but slower than pre β-lipoprotein in agarose or paper electrophoresis and it is present in the 1 050–1 080 g/ml density class upon ultracentrifugation

Pre-β_1-lipoprotein is an atypical pre β-lipoprotein which is present in a certain proportion of normal sera. It occurs relatively frequently in the sera of patients with coronary heart disease (CHD) When pre-β_1-lipoprotein is present, it occurs as a distinct electrophoretic zone in the area between β-lipoprotein and regular pre β-lipoprotein The presence of pre-β_1-lipoprotein appears t be under genetic control (4)

A study of Finnish patients with suspected or proven CHD (3) showed that a very close relationship exists between the phenotype Lp(a+) and the occurrence of pre-β_1-lipoprotein in the serum. Both phenomena were more frequent in a sample of Finnish CHD patients than in normal population samples studied previously Since both lipoprotein traits are present in a certain proportion of normal sera, they are not the results of disease They may however represent genetic risk factors with respect to CHD demonstrable early in life

The purpose of the present investigation was to study whether the two phenomena are associated also in healthy young people and to study the possible relationship between these phenomena and some other factors which may be related to the development of CHD

MATERIAL AND METHODS

Blood samples were collected from tw series of healthy young S edes from the Boden area

The first series consisted of 75 males, 16–17 years old The blood samples were drawn in the morning after 12 hours fast and centrifuged after clotting. Serum was then secured immediately chilled, stored at 4°C and subjected to electrophoretic and other clinical-chemical analyses as described previously (5) A portion of each serum sample was frozen immediat ly and transferred by air without delay in the frozen state to the Institute of Medical Genetics, University of Oslo where Lp typing was performed

The second series consisted of 53 females, 4–30 years old The blood samples from these persons were taken under les strict conditions. The samples were kept for several hours at 4°C before freezing and were stored at −20°C in Boden for several weeks before they were shipped to the Institute in Oslo for Lp typing.

Both males and females were asked to complete questionnaire about their smoking habits The females also answered a questionnaire aimed at revealing CHD and high BP among their relatives and were questioned with respect to use of oral contraceptives. Because of the young age of the subjects, family anamnesis was concentrated on disease in the omen s parents, grandparents, uncles and aunts.

Scoring with respect to the Lp(a) antigen was conducted in Oslo described previously (.). Electrophoretic lipoprotein analys and all other laboratory

Table I *Distribution of 75 young healthy males and 51 young healthy females with respect to Lp phenotype and pre β_1-lipoprotein*

		No of individuals				
		Lp(a+)	Lp(a−)	Total	χ^2	p
Male						
Lp( ) antigen and pre-β_1-lipoprotein	Pre-β_1-lipoprotein present	17		19	40.60	<0 0001
	Pre-β_1-lipoprotein absent	5	51	56		
	Total	22	53	75		
Lp(a) antigen and high amount of pre-β_1 lipoprotein	Samples w th strong pre β_1 lipoprotein band	6	0	6	12.22	<0.001
	All other samples	16	53	69		
	Total	22	53	75		
Female						
Lp( ) antigen and pre-β_1-lipoprotein	Pre-β_1-lipoprotein present	9	7	16	20.19	<0.0001
	Pre-β_1-lipoprotein absent	0	35	35		
	Total	9	42	51		

analyses wer carried out n Boden a reported previously (5). Scoring with respect to Lp(a) phenotype and presence or absence of pre-β_1-lipoprotein was carried out independently and blindly

RESULTS

Relationship between Lp(a) antigen and pre-β_1-lipoprotein

the Lp typing procedure the samples from lthy young males yielded clear-cut results ery sample being scorable as positive or negative with respect to the Lp(a) antigen. Of the 75 samples 22 gave a positive reaction The reactions with 4 of the sera were weak but clearly visible It was concluded that Lp typing in this series probably yielded valid results

Lp typing was more difficult in the series of young females Of 53 serum samples only 9 were clearly positive of which yielded very weak reactions The reactions with the remaining 7 positive samples were clearly weaker than the reactions usually observed with fresh sera Two of the 53 sera could not be assigned either to positives or negatives and the results with these were omitted in the further analyses. The fact that sera had not been treated in a way optimal for Lp typing the low frequency of positive sera in this sample the faint precipitin bands observed and the 2 doubtful reactions made it reasonable to assume that several truly Lp(a+) sera might have been typed as Lp(a−) Since it seemed reasonable to assume that the sera which still reacted positively were those which originally had been strongly Lp(a+), it was considered of interest to analyse this series of samples as well despite the fact that the Lp(a+) frequency observed in this sample might be significantly lower than the true frequency

The distribution of the 75 young healthy males and the 51 females with respect to Lp(a) phenotype and presence or absence of pre-β_1-lipoprotein is shown in Table I Among the young males scored as pre-β_1-negative there were some whose sera exhibited extremely weak and doubtful zones upon agarose gel electrophoresis. In a previous comparison with different other parameters (5) these subjects were classified as pre-β_1-lipoprotein positive

In both series a highly significant, positive correlation between the two phenomena was observed ($p<0$ 0001) Among the males except for 2 sera scored as possessing pre-β_1-lipoprotein the immunological Lp test revealed all sera with this lipoprotein and 5 more All Lp(a+) sera in the female series exhibited pre-β-lipoprotein. A visual scoring of the intensity of the pre β_1-lipoprotein zone was performed and 6 samples were scored as strongly positive with respect to pre-β_1-lipoprotein, presumably containing a high amount of this lipoprotein. Table I also shows the distribution of the 75 young males with respect to high amount of pre-β -lipoprotein and Lp(a) phenotype Again a

Table II *Lp phenotype and pre β_1-lipoprotein in oung healthy women with po itive or negative family history of CHD*

A = family history negative B = family history positi C = CHD in one of the parents
Figures within parentheses denote percentages

	A (n)	B (n)	A v. B χ^2	A v. B p	C (n)	A vs. C χ^2	A vs. C p
Lp(a+)	1/14 (7)	6/30 (20)	0 41	0.50–0.70	3/13 (23)	0.39	0.50–0 70
Pre-β_1-lipoprotein present	_/14 (14)	1_/3? (37.5)	1.50	0.20–0.30	6/13 (46)	1 93	0 10–0.20

high degree of association is obvious ($p<0$ 001) All samples scored as strongly positive with re spect to the pre β_1-lipoprotein were also positive (and exhibited strong reactions) with respect to the Lp(a) antigen

Lp phe otype and pre-β_1-lipoprotein in relation to family history of coronary heart disease

Forty-six of the females completed the questionnaire concerning familial occurrence of CHD (among them were the whose sera could not be scored with respect to Lp phenotype) Of these females 14 had a completely negative family history Thirteen reported coronary artery disease in at least one of the parents (and often in several other relatives) For the analysis no distinction was made between sustained myocardial infarction and angina pectoris Two of the females each re ported high BP in one of the parents but no evidence of myocardial infarction or CHD The remaining 17 reported one or more cases of coronary artery disease among grandparents uncles and aunts but no such disease in their parents Table II summarizes the data with respect to Lp phenotype and presence or absence of pre-β_1 lipoprotein in females with negative or positive family history The Lp(a) frequency was _8 times higher in females with a positive family history than in those with a negative and pre-β_1-lipoprotein could be demonstrated 2.6 times more frequently in those with a positive family history than in those with a negative However these dif ferences were not statistically significant.

Table II shows also a comparison between females with a negative family history and those who reported CHD in one of their parents The tendency to a higher frequency of positives for the Lp antigen as well as the pre-β_1-lipoprotein is more pronounced in this group of females who have a first-degree relative with CHD but the difference from those with a negative family history is still not significant.

Lp phe otype and pre-β_1-lipoprotein relation to use of contraceptive pills

In Table III the material of young females is analysed with respect to use of contraceptive pills and Lp(a) lipoprotein or pre-β -lipoprotein No association between use of contraceptive pills and the occurrence of the lipoprotein phenomena was revealed

Lp phenotype and pre-β_1-lipoprotein in relation to smoking

In Table IV the series of young females and males are analysed with respect to smoking habits Lp phenotype and presence or absence of pre-β lipoprotein. For the purpose of the analyses persons smoking 10 or more cigarettes per day were scored as smokers and those smoking less as nonsmokers

No association between Lp phenotype and smoking or between presence of pre-β_1-lipoprotein and smoking was found n the females Only 5 of

Table III *Lp phenotype and pre-β_1-lipoprotein in young healthy ome using or not using the pill*

Figures within parentheses denote percentages

	Not using the pill (n)	Using the pill (n)	χ^2	p
Lp( )	7/33 (21)	_/18 (11)	0 77	0.50–0 70
Pre-β_1-lipoprotein present	11/35 (31)	6/18 (33)	0 03	0 80–0 90

Table IV *Lp phenotype and pre-β_1-lipoprotein in young healthy females and males smokers or non-smokers*

Figures within parentheses denote percentages

	Smokers (*n*)	Non-smokers (*n*)	χ^2	*p*
Females				
Lp( )	[illegible]/16 (12.5)	5/28 (18)	0 002	0 95–0 98
Pre-β -lipoprotein present	5/17 (29)	9/29 (31)	0 05	0 80–0 90
Mal				
Lp( )	[illegible]/7 (29)	14/46 (30)	0 1	0 70–0.80
Pre-β_1 lipoprotein present	[illegible]/7 (29)	22/46 (48)	0.30	0 50–0 70

the 29 female non-smokers smoked 1–9 cigarettes per day The conclusions from the association tests were not altered by including those 5 in the smoking group instead of scoring them among the non-smokers ($0.80<p<0.90$ for both comparisons)

Similarly in the series of young males there was no association between smoking and either Lp(a) phenotype or presence of pre β -lipoprotein This conclusion was not altered by including in the smoker group those who smoked 1–9 cigarettes per day ($0.50<p<0.70$ for the Lp comparison and $0.70<p<0.80$ for the pre-β -lipoprotein comparison)

DISCUSSION

The results of Lp typing of the sample of young males from Northern Sweden revealed a frequency of the phenotype Lp(a+) in this sample of 29.33 % This is not significantly different from the frequency in a sample of healthy blood donors in the Oslo area ($\chi^2=0.81$ $0.30<p<0.50$) The sample of North-Swedish females is not suitable for Lp(a) frequency determination since there is evidence that some positive reactions were lost Weak reactions with sera which type as Lp(a+) and a low frequency of positive reactors are typical findings in Lp typing of sera which are not sufficiently fresh for this analysis Presumably the sera of this sample which still reacted positively were those possessing a high amount of Lp(a) antigen and this was the rationale for utilizing also this series of persons for the comparisons in the present investigation

The comparative analysis of the distribution of Lp(a) antigen and pre β_1-lipoprotein in the sample of 75 young healthy males (Table I) proves beyond any reasonable doubt that there is a strong, positive association between the presence of the Lp(a) antigen and the pre β -lipoprotein ($p<0.0001$)

Of the 7 samples which were neither positive nor negative with both the immunological and the electrophoretic techniques 5 were Lp(a+) and lacked the pre β -lipoprotein This is in agreement with the view that more positives are revealed by the more sensitive immunological technique The remaining sera were Lp(a−) but exhibited a distinct electrophoretic band in the pre β -region We have previously commented on the possible VLDL nature of electrophoretic bands in this region, occurring with Lp(a−) serum However no ultracentrifugal characterization of the lipoproteins was done in this study

This high degree of association between the two lipoprotein phenomena confirms the association observed in a sample of Finnish patients with suspected or proven CHD (3) The fact that three different samples the Finnish series and the two included in the present study have all revealed a highly significant association between the Lp(a) antigen and presence of pre β_1-lipoprotein provides firm evidence that the two lipoprotein phenomena are indeed very closely related The results clearly show that the pre β_1-lipoprotein may be identical to the distinct lipoprotein population carrying the Lp(a) antigen the Lp(a) lipoprotein.

Although the statistical analysis for a possible correlation between Lp(a) antigen and pre-β_1 lipoprotein on the one hand and a family history of CHD on the other (Table II) did not yield significant χ^2 values it seems noteworthy that the tendency in the present series is very similar to that in the Finnish patients (3) In the series of young females those with one parent with CHD presumably represented the highest degree of familial tendency to this disease When this group of females was compared with those with a completely negative family history it was striking that a 3 1 relationship was observed for the frequency of Lp(a+) as well as for presence of pre-β_1 lipoprotein (Table II) Pre β lipoprotein/Lp(a) lipoprotein is presumably closely related to the lipoprotein component observed by Knoblock and Hall (7) and their component appeared to be posi-

tively correlated with a family history of CHD Thus a trend to association with familial aggregation of CHD has been observed in total of three samples our Finnish sample (3) the present sample of young females and the sample of healthy persons reported by Knoblock and Hall Since all three series were small the conclusions must still be drawn with caution Ne ertheless the combined results do suggest that Lp(a) antigen and pre β_1-lipoprotei are positively correlated with familial aggregation of CHD These genetically determined lipoprotein phenomena may therefore constitute important genetic risk factors w th respect to CHD

As far as the present investigation goes there appears to be no correlation between the use of contraceptive pills and the presence of Lp(a) antigen or pre β_1-lipoprotein (Table III)

No association between smoking and presence of either of the two lipoprotein phenomena under study was demonstrable in the two present samples (Table IV) This finding differs from the results in a Finnish study (6) where an association between smoking and presence of pre-β_1 lipoprotein was found In the total Finnish sample no association between smoking and the phenotype Lp(a+) was demonstrable (3) When the Finnish patients who had radiologically demonstrable CHD were analysed separately there wa an association between smoking and the phenotype Lp(a+) Among those without radiologically demonstrable abnormalities there was no suggestion of an association.

The two samples analysed in the present study provide evidence that neither Lp(a) antigen nor pre β_1-lipoprotein is associated with smoking in young healthy adults The significance of the association between the two l poprotein phenomena and smoking in patients with manifest CHD is at present unknown and there is a need for further study of this apparent association It is however tempting to speculate that the association observed n the patients may imply that those possessing the Lp(a) ant gen/pre-β -lipoprotein and who are smokers run a increased risk of contracting manifest CHD

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CUTANEOUS ERYTHEMA WITH OR WITHOUT NECROSIS LOCALIZED TO THE LEGS AND FEET—A LESION IN ELDERLY DIABETICS

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Abstract The paper describes cutaneous lesions of the leg and/or foot in lderly diabetics, consisting of areas of erythema the size of child palm or larger and in the same group of patients area of necrosis from the size of confetti up to the size of child s palm surrounded by erythema. The necrosis invol es all layers of the skin. Similar ski lesions are seen in lderly persons without recognized diabetes. When group of patients of the latter type was compared with control group it appeared that their glucose tolerance was altered in diabetic direction. The skin lesions usually follow cardiac decompensation, with or without oedema of the legs, or unilateral oedema of the leg due, for example, to venous thrombosis The author suggests that the lesions are due to qualitatively or quantitatively altered reaction in diabetics. The patients may also have skeletal destruction in the feet. The initial lesion in dermopathia diabetica (Melin) are also described. They consist of superficial ulcerations the size of confetti or somewhat larger surrounded by narrow zone of erythema.

Diabetic skin lesions have been studied at the Department of Medicine in Umeå for over a decade and in 1964 Melin (48) described small atrophic, well demarcated scars on the legs of diabetics Melin s observations have been confirmed by a number of authors (3 7 14 55) and these skin lesions are now usually called dermopathia diabetica (7 9 10 11 15 21 —, 32, 38 51 64)

Another cutaneous lesion of the lower extremities apparently typical of diabetes has been observed at the Department of Medicine in Umeå. It consists of erythema of the skin of the leg or the foot. The centre of the area is often necrotic To the author's knowledge they have hardly been described earlier A close study has been made of them including an attempt to identify possible precipitating factors

Some patients with erythema and necrosis of the skin of the feet ha e been found also to have destruction of the bones of the feet The simultaneous presence of skin lesions of the type described by Melin (48) has been recorded with special attention to the initial stages of such lesions which have received little attention (22) The present paper also describes number of cases of erysipelas of the lower extremities

Erythema in connection with diabetes has been observed earlier Reddening of the face in diabetes was first described by on Norden and Isaak (59) who termed it rubeosis faciei This phenomenon was studied in detail by Gitelson and Wertheimer Kaplinski (74) Lundbæk (43) has described a condition which he calls rubeosis plantarum I describing a material of distal gangrene of the lower xtremities Ipsen (31) mentions that in warm gangrene the border of the necrotic area may be reddened Most of his patients were diabetics Ulcers of the ball of the foot, mal perforant" have long been known to occur in diabetics They have been described by Marchal (45) in 1864 Pryce (63) in 1887 and others and have been assumed to be due to neuropathy (76) Erythema of the skin of the leg in connection with arterial ischaemia has been described (40) but information as to the possible concomitant presence of diabetes is lacking. Ischaemic ulcers of the legs (17 18 30) and so-called hypertensive ulcers of the legs (47) ha e also been described without mention of whether diabetes was present or not. Skeletal destruction in association with distal diabetic gangrene with ulceration has been described (40) Destruction of the bones of the foot not obviously connected with skin ulcerations was probably first

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Table I *Data n the patients in g oups A and B*

	N	Age (y)	Precipitating factor: Cardiac decompensation	Unilateral leg oedema	Arterial insufficiency	Neuropathy	Swelling of erythematous zone	No swelling of erythematous zone	Diabetes not diagnosed
Group A									
	4	40–59	3	1	1			2	1
	33	>59	1	1	7	18	21	12	16
Total	37		15	13	8	18	23	14	17
Group B									
	7	40–59	1			1	3	4	
	37	>59	19		14	18	16	21	10
Total	44		20		16	19	19	25	10

mentioned by Jordan in 1936 (33) The skeletal destruction may heal (49 61 6_)

MATERIAL, DEFINITIONS AND METHODS

Cutaneous erythema of the leg, with or without necrosis w observed 94 patient Among these patient there were 62 with know diabetes and 32 who were not recognized diabetics Of the 94 patient 79 were hospitalized in the Department of Medicine and 15 were out-patients 14 of the latter wer known diabetics Of the 62 patients with know diabetes 1 were on insulin, 40 on oral antidiabetic drugs and 1 was treated with diet alone

The patient were ollected over period of little more tha four years in the Department of Medicine Umeå University which serves population of somehat er 100000 persons Collection of the material not entirely comprehensive The described skin s, erythema or erythema and necrosis, were all manifest when the patient were admitted to the ospital and antibiotic therapy had not been gi en prior to the admission

Known diabetes recognized diabetes or "open diabetes efer to previously recorded diagnoses of diabetes or to the presence of manifest, non-renal glucosuria at the time of examination The parts described as reddened or erythematous were as rule intensely red Swelling ys refers to welling so pronounced that finger pressure left a clear impression Neuropathy refers t the absence of Achilles and patellar reflexes Vibratory perception in the malleoli was always definitely impaired By arterial insufficiency is meant the absence of pulses in both the a. dorsali pedis and the tibialis posterior These patients nearly always also lacked pulse in the a. poplitea. I many cases pronounced oedema made it impossible to ascertain whether neuropathy or arterial insufficiency was present. In the tables these are tabulated as cases in which neuropathy or arterial insufficiency was absent. "Skeletal destruction localized to the bones of the foot refers to the skeletal lesions described in the literature (6 23 33 36 37 46, 66), demonstrated roentgenologically By erysipelas is meant ski lesions accompanied by reddening and heat as described in the literature (67) in patient with increased temperat re and in whom there was an elevated antistreptolysin titre (AST) (34) during the period of observation.

Patients with erythema on the medial aspect of the leg, with or without lceration, are not included in the material in view of the common occurrence of varicose lesions i this location.

All the patients in whom the bove mentioned skin lesions were observed were entered in a register and the lesions were photographed in colou The examination was performed with the patient in recumbent position

Patient with the skin lesion listed who were not known diabetics were investigated by an oral glucose tolerance t st in which 50 g glucose wa administered. Blood glucose wa determined by modification of the glucose oxidase method (28) with the patient in fasting tate and then every half-hour for 4 hours following the administration of glucose The blood glucose values in mg/100 ml were plotted on the ordinate of a coordinate ystem. Time in half-hour intervals was plotted on the absci sa. When the blood glucose values were connected 8 parallel trapezoids were obtained having the same height (= ½ hour) and various lengths according to the indi idual blood glucose values. The area of each trapezoid wa calculated as well as the combined area of the 8 trapezoids. The latter area represents a measure of the glucose tolerance.

The significance of the difference between means was calculated using Fischer's *t*-test. Fischer exact probability test and χ^2-analysi were also used

RESULTS

Group A

Erythema of a skin area at least the size of the palm of the hand on the anterior lateral or pos-

ration of open diabetes (y)

5–9	10–14	15–19	>19
5	1	2	
5	1		
8	5	3	4
10	7	3	6

terior aspect of the lower leg or reddening of the skin over most of the dorsum of the foot 37 patients (16 men, 1 women) with an average age of 73 years (men 71 women 74) The 70 patients with known diabetes had had the disease for on an average 5 4 years (range 1–16) (Table I Figs. 1*b* and).

Thirteen patients had erythema only on the foot 10 only on the leg, and 14 on both foot and leg Fifteen patients had bilateral erythema of the lower xtremities In 7 of the patients the duration of the erythema could be determined and varied from 5 to 22 days In these 7 patient the reddened area was wollen, the welling had always preceded the erythema by from 3 days to an indefinite time When the swelling receded the erythema alway disappeared. Of the 15 patients in whom cardiac decompensation was stated to be precipitating factor 11 had definite oedema of the lower leg.

Phlebography was carried out in 9 of the 13 patients with unilateral leg oedema and deep venous thrombosis demonstrated in 6 of them

Six (16%) of the patient had reddening of the soles of the feet.

Eight patients also had small atrophic scars on the anterior aspect of the leg, of the type described by Melin (48) Three of the patients with cardiac decompensation or unilateral leg oedema also had signs of arterial insufficiency Tw of the patients with unilateral leg oedema also had cardiac decompensation. In Table I they are included among patients with unilateral leg oedema. In one patient with recognized diabetes, widespread reddening and swelling of both feet occurred in connection with increased severity of chronic rheumatoid arthritis. In 6 of the patients no precipitating factor such as cardiac decompensation or unilateral leg oedema could be ascertained Neither did these patients have signs of arterial insufficiency Five of these 6 patients ere known to have diabetes.

The frequency of know diabetes among patients in hom precipitating factors such as cardiac decompensation, un lateral leg oedema or arterial insufficiency were recognized was 15/31 The difference between 5/6 and 15/31 is stati tically significant ($p<0.02$).

Group B

Necrotic ulceration of an area of skin ranging in size from that of confetti to that of a child s palm w th or without a crust surrounded by in tense erythema. The ulcer was located on the dorsum the lateral aspect or the sole of the foot or on the anterior lateral or posterior aspect of the leg 44 patients (16 men 28 women) with an average age of 69 years (men 74 women 65). The 34 patients with known diabetes had had the dis ease for on an average 11 years (range 1–34) (Table I Figs 1*a* and 3).

The erythematous area had lways been many times larger than the area of necrosis. The necrosis involved all layers of the skin and was nearly always painless

Of the 20 patients in whom cardiac decompensation was stated to be precipitating factor 12 had definit oedema of the lower leg Only 14 of the 44 patients were able to give an account of how the necrosi be gan In 11 it had begun as large zone of erythema in which central necrosis gradually developed in 3 it had begun as small red spot

The necrosis wa located on the foot in 26 patients (the dorsum in 5 dig. I in 7 dig II in 1 the heel in 6 the lateral border in 3 an interdigital space in 1 the sole in the egion of metatarsal I in 2, and the sole in the region of the head of metatarsal V in 1) on the lower leg in 16 and on both the foot and lower leg in 2. In the 16 patients with necrosi on the leg, the anterior posterior and lateral aspects were affected in 3 4 and 9 cases respectively Seven of the patients with necrosis on the foot and 5 with necrosis on the lower leg had multiple necroses, the largest of which is given as the main site. Nineteen (43%) patients had reddening of the soles of the feet. Nine patients also had small atrophic scars on the anterior aspect of the leg, of the type described by Melin (48)

In the cases in which it was possible to follow the clinical course the necroses usually healed, but 4 patients underwent amputation of the affected part Seven patients died of the acute illness responsible for their admission, before the necrosis had healed

Eight of the patients with cardiac decompensation or unilateral leg oedema also had sign of arterial insufficiency In 14 of the patients no precipitating factor such as cardiac decompensation or unilateral leg oedema could be identified Neither did these patients have igns of arterial insufficiency Thirteen of these 14 patients were known to have diabetes. The frequency of know diabetes among patients in whom precipitating factors such as cardiac decompensation unilateral leg oedema or arterial insufficiency were recognized was 21/30 The difference between 13/14 and 21/30 is statistically significant ($p<0.001$).

Table II *Data on the patients in group C*

	N	Age (y)	Swelling of affected part	No swelling of affected part	Neuropathy	Duration of open diabetes (y) 0–4	5–9	10–14	15–19	>19
	4	40–59	3	1	4		1	1		2
	6	>59	3	3	4	1	1	2	1	1
Total	10		6	4	8	1	2	3	1	3

Among the patients in group B the frequency of known diabetes was 77.3% and among the patients in group A 54.1% ($p=0.017$) (Table I). In the former group the mean duration of diabetes was 11.0 years and in the latter 5.4 years. Calculation of the significance of the difference between the means for the two groups yielded $t=3.20$, d.f. 52 and $0.001<p<0.005$.

Group C

Destruction of the bones of the foot demonstrated roentgenographically in patients with cutaneous necrosis on the foot. 10 patients (2 men, 8 women) with a mean age of 63 years (Table II). All of these patients are also included in group B. All were known to have diabetes and the mean duration of the disease was 17.0 years (range 3–34).

Three of the patients had widespread destruction in several of the metatarsal bones, especially in the metatarsophalangeal region, 3 had similar changes localized to the metatarsophalangeal region of dig. V, ... had destruction in the distal phalanx of dig. I and 1 had destruction of the middle phalanx of dig. II. One patient, a 65-year-old woman with diabetes of 22 years duration who had multiple ulcers on one foot, had ...ed destruction of most of the metatarsal bones, ... bones of the middle foot, the talus and calcaneus ...terally and the distal end of the right tibia. Six had skin necrosis/necroses over the site( ) of skeletal destruction. The necrotic area was always smaller than the region of skeletal destruction. The bone lesion was never exposed. In one patient the area of cutaneous necrosis was far removed from the region of skeletal destruction and in 3 the cutaneous necrosis was on the other foot.

Eight of the 10 patients had neuropathy; in the remaining 2 it could not be evaluated.

In a further 8 patients with cutaneous necroses on the foot X-ray revealed a normal bone structure and in the remaining 10 X-ray examination was not carried out. The latter 10 patients were among the first observed before the author was aware of the connection between the skin lesions and skeletal destruction. In one patient bone destruction was demonstrated first, skin lesions later.

In the cases in which the skin over the site of skeletal destruction was intact, it was not possible to determine anamnestically whether skin lesions had earlier been present.

Group D

Erythema alone or erythema and necrosis recurring after healing. 6 patients (2 men, 4 women) with a mean age of 68 years (range 56–76). One of these patients is also included in group A and five in group B.

The recurrence in four patients consisted of erythema and in two of erythema and necrosis. Recurrence did not always involve the same site as the initially observed skin lesions. The initial lesions were confined to one leg, but in three cases recurrence involved both legs. In two of these recurrence was associated with cardiac decompensation and oedema of the legs. In one of the six cases recurrence was associated with local arterial insufficiency.

The average interval between the first observed skin lesions and the recurrence was $1\,^{1}/_{12}$ years ($^{4}/_{12}$–$2\,^{1}/_{12}$). It should be noted that the time of recurrence could not be determined with greater accuracy than to the nearest month. Five patients had neuropathy and four had arterial insufficiency. All were known diabetics with a mean disease duration of 11.2 years (range 3–24).

Group E

Encrusted ulcers the size of confetti or somewhat larger, surrounded by a reddened border. 8 patients (5 men, 3 women) with a mean age of 61 years (Table III). The ulcers were located on the anterior aspect of the leg and involved only the superficial layers of the skin. The ulcers were multiple in 7 cases and arranged in a linear pattern or in groups (Fig. 4).

Seven of the patients were known diabetics with a mean disease duration of 12.4 years (range 1–29). The patient who was not a recognized diabetic had a clearly diabetic type of glucose tolerance test.

In the three cases in which it was possible to follow the clinical course the described lesions gradually developed into typical atrophic spots of the type described by Melin. All had atrophic skin lesions of the Melin type at the time of the first examination.

Group F

Patients who were not known to have diabetes and who had only erythema or erythema and necrosis;



Fig. 1a. Female, 73 years. Diabetes of 13 years' duration. In association with cardiac decompensation and bilateral leg oedema, the patient developed erythema of both legs and feet and necrosis on the lateral aspect of the right leg.

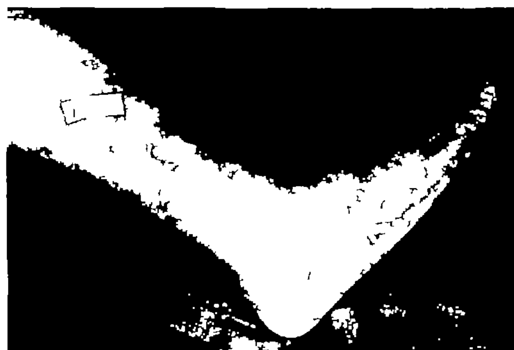


Fig. 1b. The same patient 10 months later when in association with recurrence of cardiac decompensation and leg oedema she had developed erythema of the distal parts of the legs and the feet. The areas of skin necrosis had now healed.



Fig 2. Male, 71 years. Diabetic oral glucose tolerance curve. Swelling of the right leg and foot. (Thrombosis verified by phlebography.) Erythema of the dorsum of the right foot.



Fig 3. Male, 82 years. Diabetes of 3 years duration. On the lateral border of the right foot, a small area of necrosis accompanied by widespread erythema of the entire dorsum of the foot and the distal part of the leg.

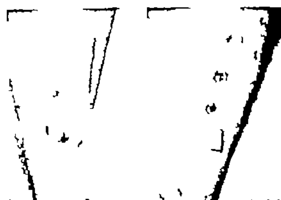


Fig 4 Male, 69 years. Diabetes of 6 years duration Multiple encrusted ulcers on the anterior aspects of both legs 7–8 months later typical atrophi scars.

28 patients (16 men 12 women) with a mean age of 71 years (men 7? women 71)

These patients underwent an oral glucose tolerance test and the results were compared with those of 35 controls. Of these 28 patients 17 are also included in group A, 10 in group B and 1 in group E.

Of the 35 controls 19 were men and 16 women with mean age of 73 years (men 73 women 71). All were hospitalized with diagnoses as similar as possible to those of the patients with skin lesions on the lower extremities not know to have diabetes. The controls were otherwise unselected and several of them proved to have clearly diabetic glucose tolerance curves.

The oral glucose tolerance tests were always per formed when the patient acute disease if any was in remission and the patient was afebrile. The results were treated as described under Material definitions and methods The glucose tolerance of the 28 patients not know to have diabetes was impaired in comparison ith that of the 35 controls. The mean values for glucose tolerance calculated in the first described manner wer 569 and 456 respectively The average mean errors were 21.3 and 16 5 respectively Calculation of the significance of the difference between the means for the two groups yielded $t=3.93$ d.f $=61$ and $p<0.001$ Comparison of the values for the controls with those of the 17 patients with erythema alone and of the 10 with erythema and necrosi yielded $t=3.25$ d.f $=50$ $0.001<p<0.005$ and $t=$ 67 d.f $=43$ $0.01<p<0.02$ respectiv ly

Group G

Erysipelas 5 patients (one 54-year-old man, 4 women with a mean age of 55.3 years) One woman aged 67 was known to have had diabetes for four years the other patients were not known diabetics

All had initially an oral temperature of 39 0–39 9° and an elevated AST (400–1600 U). All had uperficial ulcers, deep ulcers or fissures of the ski adjacent to the reddened area which in three cases involved the foot and leg in tw cases only the leg. I four patients the affected leg was markedly swollen. None had car diac decompensation.

The glucose tolerance tests revealed no ignificant difference between these patients and the controls ($t=1.38$ d.f $=37$ and $0.10<p<0.20$)

DISCUSSION

The skin lesions described characterized by erythema or erythema and necrosis are localized to the distal part of the lower extremities They occur n persons especially in the elderly with recognized diabetes, but are also see in elderly persons not known to have diabetes However comparison of the glucose tolerance test values for a group of the latter type of patients with those of a control group showed a clear alteration in the diabetic direction. The difference was statistically highly significant. In other words the skin lesions are closely connected with diabetes

The skin lesions are usually precipitated by cardiac decompensation with or without oedema of the legs or by unilateral leg oedema due for example to venous thrombosis (Table I) They may possibly be precipitated by arterial insuff ciency

Table III. *Data on the patients in group E*

	N	Age (y)	Neuro-pathy	Swelling of affected part	No swelling of affected part	Diabetes not diagnosed	Duration of open diabetes (y) 0–4	5–9	10–14	15–19
	2	20–39	2		2					
	1	40–59			1	1				
	5	>59	4	1	4		1	3		1
Total	8		6	1	7	1	1	3		3

Entmacher et al (70) state that cardiac disease is 2 0 times as frequent a cause of death among diabetic men as among men in general The corresponding figure for women is given as 3.. Of the precipitating factors mentioned above cardiac decompensation would therefore be expected to be more common among diabetics than in the population at large Whether unilateral leg oedema is more common among diabetics than in the general population is not known In Sweden the frequencies of recognized diabetes in the age groups 60–69 and 70–79 respectively are 6% and 4.3% for men and 3 3% and 4.2% for women (54)

Since the factors precipitating the above mentioned skin lesions can be assumed to be .0 and 3 2 times more common among diabetic men and women respectively than in the population at large the author s finding that about 53% of the men and about 76% of the women with such skin lesions have open diabetes suggests that the development of such lesions reflects a quantitatively or qualitatively altered reaction to the precipitating factors on the part of diabetics Whether this altered reaction is due to an altered metabolism immunoreaction included in the cells of the diabetic tissue or whether it is due to an altered blood supply of this tissue which may cause decreased transport of oxygen to the tissue or of metabolites from the tissue or both cannot be decided

Among the patients with erythema and necrosis frequency of known diabetes was 77.3% and ong those with erythema alone 54 1% The rmer group differed from the latter in having a longer duration of diabetes (11 0 years compared to 5 4 years) In both cases the difference was statistically significant Erythema and necrosis must be regarded as a more powerful reaction than erythema alone Thus open diabetes and a long duration of the disease apparently predispose to a more powerful reaction to precipitating factors

It is striking, in regard to both erythema (group A) and erythema with necrosis (group B) (Table I) that precipitating factors in the form of cardiac decompensation, unilateral leg oedema and possibly arterial insufficiency were statistically significantly more common among patients not known to be diabetics than among those with open diabetes This may mean that the assumed alteration in mode of reaction is less pronounced in patients of the former type and that in these patients the skin lesions usually develop only in response to a more powerful precipitating factor

The author has not found any connection between the skin lesions discussed and arterial hypertension

The initial development of erythema alone was followed in a number of patients with oedematous swelling of the part in question It was obvious that the swelling always preceded the development of erythema and that the latter receded when the swelling subsided (compare the description of group A) The skin lesions were usually reversible Even necroses healed in over half of the patients. Exact figures are lacking since some of the patients died during the period of observation in others the affected extremity was amputated and for some patients complete clinical follow-up was impossible

It is characteristic that the skin lesions may recur after once having healed may be at a site other than that at which they were first observed. It is striking that all the patients with such recurrences had known diabetes of long duration (compare the description of group D)

X rays of the feet of patients with skin lesions often revealed skeletal destruction (group C Table II) These patients were all known diabetics with a long mean duration of the disease The skin over the sites of the bone lesions may be either intact or altered

There is probably no clear distinction between cutaneous erythema and necrosis and what is usually termed distal diabetic gangrene localized, for example to the toes or heel Of the 44 patients in group B three had obvious distal black diabetic gangrene involving the heel or the entire circumference of one or more toes It has long been known that distal gangrene of the lower extremities is common in diabetics (5)

Diabetics have often been reported to have good pulses in spite of the presence of gangrene of the toes In comparison with non-diabetics with atherosclerotic vascular foot lesions which usually have intermittent claudication and absence of pulses in the a. poplitea and the a tibialis posterior (50) In only a few of the patients in the present study with erythema or erythema and necrosis localized to the distal part of the lower extremities were the peripheral pulses absent (Table I) In most patients the skin of the lower leg was warm and there were good pulses in the

a. dorsalis pedis the a tibialis posterior and the a. poplitea. Only a few of those who lacked peripheral pulses had cold extremities or intermittent claudication The clinical significance of absent pulses or demonstrable arterial occlusion is however not clear (40). Widmer et al (68) in an investigation of 6400 industrial workers performed angiography in 83 persons and demonstrated 99 peripheral arterial occlusions in 75 of them. Two-thirds of these persons were entirely free of symptoms

The investigations of the circulation in the lower limbs of diabetics (1., 35 53 58) fail to support the assumption that arteriosclerotic lesions and lesions in the smaller vessels have any obvious effect on the blood supply to the legs of diabetics with disease of long duration even under conditions of muscular work It appears to be an open question whether the blood supply to the feet and toes is impaired in diabetics In patients with distal gangrene in association with occlusion of the chief arteries of the leg ^{133}xenon clearance studies have shown that the blood flow through the skin on the dorsum of the foot is diminished (39). Data on diabetics are lacking

Some earlier authors (4 16 42) consider that gangrene of the distal extremities in diabetics is due to arteriosclerosis In discussing the incidence of arteriosclerosis a distinction should be made between calcification of the media (Mönckeberg) and lesions of the intima (atherosclerosis) The former does not usually affect the arterial lumen (41) and is generally accepted as being more frequent among diabetics but many authors (29 44 65) have regarded it as lacking in clinical significance On the other hand it is controversial whether intimal lesions are more common among diabetics than among non-diabetics (8 25 57).

Skeletal destruction in the feet of diabetics has been attributed to neuropathy by a number of authors (1 2, 13 27 33 36 37 46 56 66) Diabetic neuropathy is especially apt to involve sensory fibres (19) Although patients with skeletal destruction always have neuropathy (6) there is no convincing evidence that the latter is the cause of the skeletal lesions (60 61)

As pointed out above there is no clear distinction between the skin lesions in diabetics characterized by cutaneous erythema and necrosis and distal diabetic gangrene The author has further ascertained that the skin lesions are usually precipitated by cardiac decompensation, with or without leg oedema or by unilateral leg oedema due for example to venous thrombosis, and has suggested that their development depends on a quantitatively or qualitatively altered mode of reaction in diabetics as opposed to non-diabetics. The hypotheses regarding the cause of distal diabetic gangrene presented in the literature do not appear to be based on convincing evidence (13)

Superficial encrusted ulcers the size of confetti or somewhat larger surrounded by reddened margins were observed in 8 of the present patients The ulcers were localized to the anterior aspect of the leg were multiple and arranged in a linear pattern or more irregularly in groups All patients with such lesions were diabetics In the three patients in whom the clinical course of the lesions could be followed they developed into skin lesions of the type described by Melin (48)

Both the skin lesions described in the present study and by Melin occur almost exclusively in diabetics and are virtually confined to the leg In the cases in which the initial stage of the skin lesions described by Melin were observed they consisted of confetti-sized ulcers surrounded by a marginal zone of erythema The lesions described in the present study (Table I) also include erythema A further common feature is that the lesions may heal and then recur It should be added that the lesions described by Melin may also be observed in younger persons with long-standing diabetes Thus there are similarities between the two types of lesions.

The present author has suggested that the skin lesions described are due to qualitative or quantitative differences in mode of reaction in diabetics as opposed to non-diabetics The fact that these lesions and those described by Melin are confined to the lower leg may be due at least in part to the fact that the proposed alteration is confined to or more pronounced in the lower extremities This assumption is supported by the following observations

In one of the diabetic patients in group E (Table III) the author elicited erythema of the skin on the back of the hand and on the anterior aspect of the leg by means of a pencil of solid carbon dioxide 8 mm in diameter On the leg the erythema developed into an ulcer surrounded by reddened zone (Fig 5a) The ulcer eventually gave rise to an atrophic scar of the type described by Melin

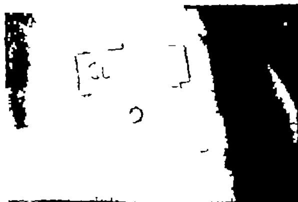


Fig 5a Male 28 years. Diabetes of 1 years duration. Ulceration and erythema of the anterior aspect of the right leg following traumatization with pencil of solid carbon dioxide 6 days previously

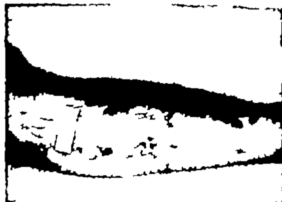


Fig 5b The same patient 8 months later The final result of traumatization is the second spot distal to the figure 36.

(Fig. 5b) The patient already had a number of such scars on the anterior aspect of the leg On the back of the hand the erythematous reaction disappeared without a trace In a similar experiment in a control person erythema disappeared without sequelae on both the leg and the hand

The skin necroses on the legs and feet described in the present paper are always surrounded by a reddened zone In the author s experience distal gangrene of the lower extremities in diabetics is also characterized by the presence of such a zone The author has observed one patient an 81 year-old woman with diabetes of long duration, who developed acute arterial circulatory insufficiency in the distal part of the right arm with slight swelling of the lower arm and a threat of gangrene of the fingers The cause was probably arterial emboli There was never any zone of erythema adjacent to the region threatened by gangrene

On the basis of appearance alone it may be difficult to distinguish the skin lesions described here—cutaneous erythema and erythema accompanied by necrosis—from erysipelas of the leg However the patients with the described skin lesions usually are afebrile and have neither increased ESR nor leucocytosis Such findings when present, have always been attributable to another acute disease In doubtful cases AST has been determined the values have always been normal In the course of this investigation 5 patients were encountered who fulfilled the earlier criteria for the diagnosis of erysipelas (group G) Among those in this group who were not known diabetics the oral glucose tolerance test revealed no statistically certain difference from the controls.

At present clinical observations on lesions in the lower extremities of elderly diabetics can be summarized under eight fairly well defined categories. 1) Distal gangrene In the author s experience characterized by a marginal zone of erythema Aetiology unclear 2) The skin lesions described by the author Localized to the lower leg or foot and characterized by erythema and/or necrosis They are usually precipitated by cardiac decompensation, with or without oedema of the leg or by unilateral leg oedema due for example to venous thrombosis Both erythema and necroses usually heal but the lesions may recur The distinction from distal diabetic gangrene is not a sharp one 3) Rubeosis plantarum (43) 4) Dermopathia diabetica (48) The skin lesions begin as superficial ulcers the size of confetti or slightly larger surrounded by a reddened margin. They develop into atrophic scars which also show increased vascularization. The lesions may heal and later recur 5) Necrobiosis lipoidica diabeticorum. Rare especially in elderly diabetics (57). 6) Peripheral neuropathy Aetiology unknown 7) Arthropathia diabetica (Charcot joints). The patients always have peripheral neuropathy and the joint lesions are generally regarded as secondary to the neuropathy 8) Skeletal destruction localized to the bones of the feet. The skin over the affected part is often intact. The destructions may heal These patients always have peripheral neuropathy

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CONGENITAL ABSENCE OF PORTAL VENOUS SYSTEM IN A 50-YEAR-OLD WOMAN

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Abstract A 50-year-old woman is reported who had no portal vein at autopsy but otherwise the liver histology and function appeared unremarkable. Retrospectively it could be assumed that she had in fact had episodes of portal encephalopathy

In 1793 Abernethy (1) described the autopsy findings of a girl with "vena portarum terminating in the inferior caval vein. Kiernan (3) in 1833 reported a similar anomaly in a 13-year-old girl who died from injury to the head and in 1954 Hellweg (2) described the autopsy findings of a 5½-month-old girl with congenital absence of the terminal portion of the portal vein.

The present paper describes the clinical history and necropsy findings in a 50-year-old woman with a similar agenesis of the portal venous system and discusses the possible effects of this anomaly on her cerebral function

CASE REPORT

The patient was considered to be in good health, including a normal pregnancy when she was 25 years old. She had an appendectomy when she was 39 and subsequent intestinal obstruction caused by peritoneal adhesion when she was 42 years old. Starting at about 40 years of age she noticed a progressive tiredness and dyspnoea.

An examination at the age of 42 suggested an atrial septal defect. But at thoracotomy which was performed acutely because of pericardial tamponade produced during attempted left-sided angiocardiography no septal defect was demonstrable. Pulmonary hypertension was demonstrated at cardiac catheterization and lung biopsy was performed. This displayed diffuse, almost completely interstitial fibrosis. It was concluded that the patient probably had an early Hamman-Rich syndrome. Because of slight hyperbilirubinaemia (1.2 mg/100 ml) liver biopsy was performed and was considered normal. There was no history of venous thrombosis, but since pulmonary embolism could not be definitely excluded she was treated with dicoumarol during the subsequent 5 years. For the remainder of her life she was incapacitated by dyspnoea, and X-ray examination of the lungs showed progressive widening of the pulmonary trunk and main branches. Liver function tests were performed on several occasions and showed maximum bilirubin of 1.1 mg/100 ml and normal levels of transaminases, alkaline phosphatases and thymol turbidity

Beginning at the age of 44 the patient was repeatedly admitted to the hospital because of drowsiness, tremor and unsteady gait. Since urinary analysis showed high concentrations of barbiturate and meprobamat and the symptoms disappeared after hospitalization, it was concluded that they were attributable to drug abuse

The patient constantly complained of tiredness and weakness in the extremities and when she was 45 she was again hospitalized. Her symptoms, which disappeared after day were attributed to overdosage of diazepam. The muscular weakness continued to be prominent problem and she complained that on occasion she was so tired that she "fell out of the chair when sitting". She denied further consumption of sedatives, and her only known medications were digitalis, diuretics, potassium and dicoumarol. At the age of 46 she was noted to have hand tremor, vertigo in the upright position and slow speech and was described as very dull. Routine neurological examination was otherwise unremarkable with the exception of bilateral weakness in arms and legs. An EEG showed slow waves bilaterally without focal changes. A second EEG when she was 47 displayed progressive abnormality but still without difference between the two sides. There was practically no activity within the α-domain.

Hypercapnia, hypokalaemia and hypothyroidism were excluded causes of her neurological symptom. Her vertigo became more prominent. It was described as non-rotatory and was not accompanied by any hearing loss, difficulties of vision or paresis. Her speech was now described as slurred. Intermittently there was also inversion of the sleep rhythm. However it was repeatedly noted that there was no disorientation. No further deterioration was noted until at the age of 50 when she was admitted to the hospital with pulmonary oedema which did not respond to treatment





Fig 1 Microphotograph of liver showing absence of portal ei and abu dance of hepatic arteries. Elastin+ van Gieson. (*a*) ×12, (*b*) ×216.

At autopsy there was a marked hypertrophy and dilatation of the right entricle The right auricle was filled with mural thrombi. There was no septal defect. The whole pulmonary tree was greatly dilated with largest circumference of the pulmonary trunk of 22 cm. The wall of the pulmonary arteries was thin and friable There were several pencil-sized endothelium adherent pulmonary emboli, several pulmonary infarctions and pulmonary oedema. Femoral azygos, and caval vei s were normal.

The superior mesenteric and the splenic eins united normally but nstead of extendi g into the liver the portal vein terminated in the inferior caval vein just above the renal eins The hepatic ins, which appeared small terminated normally the inferior caval vein The coeliac xis branched normally to the hepatic rtery which wa of about twice the normal size The er weighed 1100 g and had a smooth surface and ded edge The cut surface showed exaggerated lar architecture At microscopical xamination no intrahepatic branches of the portal veins were recognized in the portal areas which usually contained several arterial essels (Fig 1 and *b*)

The central nervous ystem was unremarkable macroscopically but in the right posterior cerebellar fossa, about 5 cm to the right of the fal cerebelli there was a meningeoma, about mm thick and about 1½ cm in diameter No further anomalies were detected on macroscopic examination

DISCUSSION

Portal encephalopathy has only occasionally been reported in patients without liver disease but with appreciable portal systemic communications due to extrahepatic obstruction of the portal vein (5) It is therefore of interest to discuss whether the patient reported here suffered from portal encephalopathy during her last years

Her neuropsychiatric symptoms (tiredness hand tremor drowsiness inversion of sleep rhythm, slow and slurred speech) are all commonly encountered in portal encephalopathy (4) The vertigo which was a prominent symptom is not a characteristic symptom of portal encephalopathy but may well have been caused by her meningeoma However the latter could not reasonably ha e caused the other neuropsychiatric symptoms Though there is no specific EEG pattern for portal encephalopathy other causes of the patient s EEG pattern such as hypoglycaemia uraemia hypercapnia and grave electrolyte disturbances could be excluded Overdosage of sedatives could theoretically have contributed to the clinical picture at the time of hospitalization and probably did so on the first admissions for tiredness and drowsiness but later sedatives were not prescribed Furthermore the EEGs were registered after several days of hospitalization when she did not receive any sedatives Apart from a slightly elevated serum bilirubin concentration there were no biochemical criteria for hepatic dysfunction, and the liver morphology was normal except for the absence of portal veins and the abundance of hepatic arteries It thus seems probable that the neuropsychiatric symptoms in this woman were caused by the portal blood by passing a liver without hepatocellular failure

Since these symptoms did not appear until after her cardiopulmonary function had deteriorated because of pulmonary embolism pulmonary hypertension and lung fibrosis it seems likely that this impairment in some way was of importance for the development of the portal encephalopathy

Hypoxia CO_2 retention alkalosis and hypokalaemia were all excluded as contributory factors However t cannot be ruled out that a progressively increasing right heart failure w th congestive liver damage could have been of importance for the development of the portal encephalopathy

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HYPOGLYCEMIA CAUSED BY AN INTRATHORACIC TUMOUR

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Abstract A patient with large intrathoracic tumour associated with hypoglycemic attacks and whose fasting glucose levels were f equently below 30 mg/100 ml is described. The immunoreactiv insulin (IRI) level du ing the hypoglycemic attacks and fasting was less than 5 μU/ml The oral glucose tolerance test was normal. Glucagon gave a subnormal increase in blood glucose and no increase in serum IRI levels Diazoxide did not reduce the frequency of attacks. The hypoglycemic attacks subsided after removal of 5 kg cell-rich mesenchymal tumou which was probably neurofibroma. The IRI concentration in the primary tumour extract was 0.05% of that found in extract from normal pancreatic tissue and only 0 0005-0.1% of that reported for insulinomas On Sephadex LH gel filtration the low IRI activity was not eluted as insulin or proi sulin All findings support the view that the hypoglycemia was not caused by insuli production by the tumour Other possible causes are discussed.

Insulinoma induces hypoglycemia by causing high serum insulin levels through insulin release The insulin content n tumour cells varies widely and is probably dependent upon the storage capacity of the cells for insulin (6)

Hypoglycemia may occasionally be associated with different extrapancreatic tumours (1 — 3 4 5 10 11 13 14 15 16 17 19 21) e.g. hepatoma, suprarenal tumours tumours originated from appendix or ovaries different epithelial tumours and f nally tumours of mesenchymal origin as first described by Doege (7) and Potter (18) The mechani m whereby such extrapancreatic tumours cause hypoglycemia, and particularly whether such tumours produce insul n is still under dispute

In the present work a patient with a large intrathoracic tumour probably of the Doege and Potter type was nvestigated. Evidence is presented which suggests that insulin productio by the tumour did not cause the hypoglycemia. Other ex planations are discussed

CASE REPORT

The patient, woman born in 1926, had previously been in good health I 1967 she developed marked hypertrophic osteoarthropathy I 1969 butterfly exanthema developed in her face and the diagnosis of LED w made in spit of negative laboratory test for this disease, however treatment with prednisone and azathioprine was initiated

During 1971 she complained of dyspnea during xer tion nd in Ja 1972 also when resting Figs 1 *b* and show the development of an intrathoracic tumou which, in retrospect, could ha e been noticed on X rays already in 1959 The tumour growth as slow until 1970 and had been falsely diagnosed as an elevation of the right diaphragm I Jan. 197? the right lung was totally collapsed by huge tumou which entirely filled the right side of the chest causing a marked displacement of the mediastinum The tumou was show by aortography to be supplied by bronchial arteries

Blood glucose homeostasis

I June 1971 the patient experienced slight fainting attacks, transient hemiplegia and dysphasia lasting for hours. She felt that these attack were relieved by taking prednisone tablet and the dosage of prednisone wa increased from 15 mg each alternat day to 20 mg daily No further attack occurred until Jan 1972, when admission to hospital became necessary due to her ex periencing recurrent fainting attacks and a diagnos of hypoglycemia was then made

Fig shows that hypoglycemia frequently re corded in the fasting state and rarely during the day The oral glucose tolerance test (1 g glucose/kg b wt) was normal.

The serum immunoreactiv insuli (IRI) levels during hypoglycemic attacks and during fasting determined on nine different occasions were all les than 5 μU/ml serum. Glucagon (1 mg) gave no insuli release and only slight increase in the blood glucose levels (Fig. 3) Diazoxide 100 mg twice daily had no influence on the attacks or on the blood sugar levels No antibodies t insulin were found in serum from the patient (8)

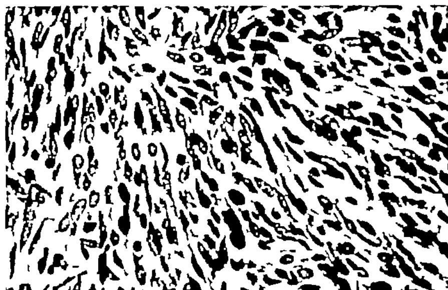


Fig 4 Histology of the intrathoracic tumour consisting of cell-rich tissue of spindle cells running in whirls suggesting neurofibroma (magnification, × 256).

that the small amount of insulin seemingly detected in the primary lung tumour extract had different properties The substance might therefore not have been insulin and one could speculate as to whether other components were present These could have possessed the same antigenic determinants as insulin they might be modified insulin or insulin attached to some substance causing different chromatography characteristics

Previous reports have given widely different results as to insulin content in similar tumours. gh insulin-like activity has frequently been meas- d by bioassays (1 2 3 4 11) The more spe ic immunoassays have usually shown absence or small amounts of insulin similar to our findings (1 4 5 11 13) The extraction methods differed however and it is suggested here that substances other than insulin which ha e the same immunoreactivity could ha e been extracted in variable amounts Volpe et al (1) suggested a similar explanation although tumour extracts were not examined

The low preoperative insulin levels in blood even after β-cell stimulation are remarkable and similar to those found in other studies (4 5 11 1 ?1) The prolonged absence of stimulation prior to the test is not a likely explanation because the patient s blood glucose increased normally after meals (Fig 2) This supports the view that such tumours apart from being large glucose consumers (4 5 11 12) also affect glucose regulating mechanisms (4 5 10 11) The low insulin levels also argue against a β-cell stimulator being secreted by the tumour

Another possible mechanism of hypoglycemia is increased glucose consumption by such large tumours There is also evidence of ineffective glucose utilization in the glycolytic pathway (4 5 11) Decreased glucose output from the liver as demonstrated by the glucagon test may also have contributed to hypoglycemia For a while prednisone counteracted hypoglycemic attacks in our patient probably because of its diabetogenic effect.

An association with other endocrinopathies in the Doege and Potter syndrome has been reported thus thyrotoxicosis has been found in 10% of cases (11) The non-functioning thyroid adenoma in our patient may be accidental

The only effective treatment of the Doege and Potter syndrome has been found to be surgical removal of the tumour (11) In spite of the large size of such tumours there is slight tendency to metastasis However recrudescence of the tumour may occur many years later as reported in the Doege and Potter case

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Announcements

The postgraduate course and workshop Progress in fibrinolysis organized by European Thrombosis Research Organization (ETRO) and Fondazione Giovanni Lorenzini will be held in Milan Italy March 6–8 1975

Fees Postgraduate course US $ 50 Postgraduate course and workshop US $ 100

Registration and information before Jan 31 1975 to Fondazione Giovanni Lorenzini Via G Lorenzini 7 (ex Via Brembo 65) 201 39 Milan, Italy

The third European Conference of Internal Medicine organized by the European Association of Internal Medicine (A E.M I E) presided over by Professor E. F Pfeiffer Ulm University will be held in the Hague The Netherlands May 1–3 1975

Topics Tropical diseases in Europe Thrombosis in internal medicine (new concepts in pathogenesis and therapy) General problems of internal medicine

Applications The Secretariat of the A E.M I E. Dr J Dagnelie rue Corsal 6c B-5861 Cortil-Noirmont, Belgium

International Conference on Prostaglandins will be held in Florence Italy May 26–30 1975

Co-chairmen Prof B Samuelsson and Prof Paoletti.

Information The Secretariat of the International Conference on Prostaglandins Dr G C. Folco *Institute of Pharmacology and Pharmacognosy* University of Milan Via Andrea del Sarto 21 201 29 Milan, Italy

The fourth biennal course on current methods of immunologic research and diagnosis will be offered by The Center for Immunology of the State University of New York at Buffalo In July 1975

Deadline for applications March 31 1975

Further Information James F Mohn, MD Director The Center for Immunology State University of New York at Buffalo Buffalo New York 14214 USA

Digestion of milk A prize of Sfr 15 000 will be awarded in Sept 1975 by the Central Union of Swiss Milk Producers, Berne to a scientist (chemists physicians biologists who have worked along this field) from the following countries. Argentina, Australia Austria Belgium Brazil Bulgaria, Canada, Czechoslovakia, Denmark Finland France India Ireland Israel Italy Japan Kenya, Luxemburg The Netherlands, New Zealand Norway Poland South Africa Spain Sweden, Switzerland United Kingdom USSR West-Germany

Applications Professor M. Demole Unité de Diététique Hôpital Cantonal CH 1 11 Genève 4, Switzerland until Jan 31 1975 Applications should be accompanied by 3 copies of a) curriculum vitae b) list of works c) reprints of or 3 papers on the theme of the prize, published in the last 5 years (no typewritten papers). These documents should be written in English French or German or should be accompanied by a translation into one of these 3 languages (*will not be* returned to the authors)

Acta med scand Vol 196 pp 353–354 1974

EDITORIAL

THE PHILADELPHIA CHROMOSOME AND CHRONIC MYELOCYTIC LEUKEMIA (CML) —STILL A COMPLEX RELATIONSHIP?

Except for a few reports of the presence of the Philadelphia (Ph¹) chromosome in rare cases of non-CML myeloproliferative diseases (18) it still holds true that the Ph chromosome is exclusively associated with CML. But what does this relationship mean in terms of etiology and pathogenesis of the disease? Although these questions have so far not been sufficiently answered recent research has thrown some more light on the Philadelphia problem.

Who has the Ph chromosome?

About 85% of the patients with CML have this abnormal chromosome in myelopoietic thrombopoietic and erythropoietic precursor cells (17) A small number of patients are mosaics for Ph positive and Ph negative cells, and the remaining 10–15% lack the abnormality (17)

The Ph chromosome does not generally disappear from the bone marrow during conventional treatment with busulfan (16). However intensive treatment leading to hypoplasia of the bone marrow may eradicate the Ph positive cell population temporarily (2, 4). Such "overtreatment" has induced long-term remissions in single patients, but has so far been considered more risky than beneficial (6). However the access to new cytotoxic drugs may change this view and the goal may become to eradicate the Ph positive cell population.

Which chromosome is affected?

For several years after the discovery of the Ph chromosome (14) it was not possible to distinguish between the two G chromosomes, i.e. numbers 21 and 22, and it was generally believed that the Ph¹ chromosome was a number 21 chromosome. Thus, it was assumed that the Ph abnormality and the G trisomy observed in Down's syndrome, condition with well known high risk for leukemia development, affected the same chromosome pair I 1970 it became clear using the Q-banding technique that the Ph chromosome and the extra chromosome in Down's syndrome were not the same (1). The Ph¹ chromosome was assigned to number 22 chromosome although the normal chromosome 22 will now with this designation be slightly longer than 21

Up till most recently the Ph chromosome was regarded as a deletion of the long arm of chromosome 22, although it was suspected for long time that it might be the result of translocation. In 1973 it was demonstrated (15) that it is in fact the result of translocation between chromosomes 9 and 22. The missing part of the long arm of the Ph was found to be located on the long arm of one of the number 9 chromosomes (15). This finding has rapidly been confirmed by others (6) A shown in this number of the Acta Medica Scandinavica (8) Ph¹ negative patients with CML do not show the additional chromosome material on the long arm of chromosome 9 (or on other chromosomes) which provides further evidence that the extra material on chromosome 9 in Ph positive patients is translocated from 22. It was initially believed that this translocation was the only one associated with Ph¹ positive CML. However most recently it was found in three patients that the fragment from chromosome 22 could be translocated to other chromosomes i.e. chromosomes ., 19 and the homologous 22 (5 10, 12) without apparent differences in the clinical hematological pattern or course of the disease (8, 10). In fourth patient the missing chromosomal fragment on 22 was not found on any other chromosome (13). However translocated fragment may have been undetected for technical reasons and it is not clear that this case is an example of a true deletion. Thus, it appears that the Ph¹ chromosome is most frequently the result of translocation between 9 and 22, but may also rarely occur as result of translocation between 22 and other chromosomes (Fig. 1).

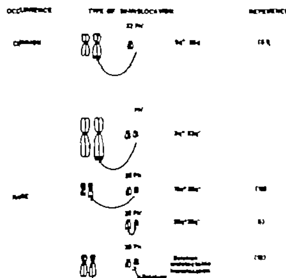


Fig 1 Variant translocations in CML.

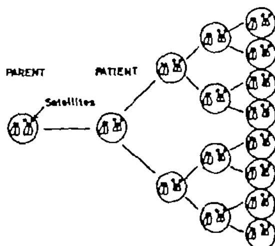


Fig 2 Clonal development of CML. Normal chromosomes 22 in parent cells. Ph acquired through translocation in one of the patient's stem cells committed for myeloid, erythroid and thrombopoietic cell differentiation. After multiplication Ph only in the satellit carrying chromosome 22 of leukemic cells.

Is the Ph chromosome acquired? If so in which chromosome 22—the one from the father or the one from the mothe

It is well known that the Ph abnormality is acquired since it does not exist in myelopoietic cells in neither an identical twin (11) nor in parent of Ph positive CML patients (7 9). Furthermore, it can be acquired from either the maternal or paternal chromosome 22 (9). This could be shown sing fluorescent satellites on chromosomes 22 as markers 2)

Does the abnormal ell population develop from a mutation in single cell or from simultaneous mutations in man ells

During the satellite study mentioned above it was found that the Ph abnormality was always found in the satellite marked chromosome 22 (Fig 2). In one patient the Ph chromosome was found in all metaphases in the satellite marked 22 received from the mother and in the other from the father strongly supporting the view that the Ph positive cell population has developed from one single cell (clonal origin). The only argument against such a view would be an inherited predisposition in the respective parental chromosomes for development of the Ph^1 abnormality Although unlikely such a predisposition could result in either a multicellular or unicellular (clonal) development. There is further evidence in favour of clonal origin, i.e. the fact that granulocytes from females with CML and heterozygosity for glucose-6-phosphate dehydrogenase variants possess only one enzyme variant (3).

Unresolved problems?

There are many more problems to be resolved about CML and the Ph chromosome, i.e. how is the translocation induced? What are the etiologic agents inducing it? Is it a virus? Is it a different virus for different translocations? Furthermore and most important—is the Ph^1 chromosome only the result of the disease or is the translocation the first step in its development? Hopefully these and many more questions about the Ph and CML, which are of importance for the understanding of the disease and thus ultimately for the treatment of the patient, will be resolved in the future.

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INVOLVEMENT OF CHROMOSOMES 8 9 19 AND 22 IN PH[1] POSITIVE AND PH[1] NEGATIVE CHRONIC MYELOCYTIC LEUKEMIA IN THE CHRONIC OR BLASTIC STAGE

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Abstract Chromosome 9 had additional chromosomal material at the end of the long arm in 15 of 16 patients with Ph positive CML. The remaining patient had a normal chromosome 9 but additional material on 19 The amount of additional material was approximately the same as that lacking on chromosome 22. Translocations between 9 and 22, and 19 and 22, respectively are therefore suggested. One patient with Ph[1] negative CML had no detectable additional chromosomal material on any chromosome including chromosome 9 which gives further support to the view that ther is indeed a translocation in Ph positive patients. Extra chromosomes appeared in two of three Ph positive patients in the blastic stage. Both patients had extra number 8 chromosomes and double Ph and one patient had further extra chromosomes, i.e. 7 11 12, 16, 17 and 19

It has recently been shown using the Q-banding technique (6) that one of the number 9 chromosomes has additional chromosomal material on its long arm in chronic myelocytic leukemia (CML) (20) The amount of additional material has in most cases been approximately the same as that lacking on the Ph chromosome previously identified as number 22 (3) It has therefore been suggested that there might be a translocation between chromosomes 9 and 22 in CML. Only four patients with Ph positive CML and normal chromosomes 9 have so far been described However additional chromosome material was found in three of these patients on other chromosomes (9 12, 16).

In the present work we also report one patient with Ph negative CML, in whom there was no additional chromosome material on chromosome 9 or on any other chromosome a further proof of the view that there is in fact a translocation in Ph positi e CML.

An extra chromosome 8 appears to be associated with premalignant or malignant hematological diseases engaging myelo- or erythropoietic cells (7 17 18 21). From the present work it appears that the extra C group chromosome sometimes found in the blastic stage of CML may frequently be a number 8 chromosome

PATIENTS

Seventeen patients with CML were investigated cytogenetically Eight of the patients were studied before treatment and also during busulfan therapy Most of the patients who were on busulfan treatment were analysed at stage of insufficient treatment and thus at a relatively high WBC. The relevant hematological data at the time of investigation are seen in Table I.

Fourteen patients were at chronic stage of the disease one of whom (no. 14) was also investigated in the blastic phase All these 14 patients were Ph positive. Two further Ph positive patients (nos. 15 and 16) were investigated in the blastic phase of the disease. One Ph negative patient (no. 17) was investigated shortly after institution of treatment, but was apparently already in an early blastic phase (Tables I and II).

METHODS

Bone marrow and blood specimens were investigated in all patients. Chromosome preparations from bone marrow specimens were made directly on fresh aspirates as described previously (24) and from conventional peripheral blood cell cultures after 72 hours incubation.

The Q-banding technique was performed as described previously (6). Metaphases were selected and photo-

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Table I *Hematological data at the time of chromosome analysis*

Pat. no	Age (y)	Sex	Previous treatment	Hb (g/100 ml)	Platelets $\times 10^{-3}$/mm^3	WBC $\times 10^{-3}$/mm^3	Immature cells (%)	Blast cells (%)
1	34	♂	None	11.0	509	164 0	27	5
2	29	♂	None	12.0	120	167 0	37	3
3	40	♂	None	9.2	728	148.0	37	4
4	38	♀	None	7.8	690	86.8	37	8
5	34	♀	None	14.4	422	70 6	25	1
6	44	♀	None	13.9	1 064	20.9	1	0
7	79	♀	None	13.8	1 730	24.5	5	0
8	63	♂	None	12.8	1 200	26.4	3	0
9	19	♀	Busulfan	13.5	388	31 1	14	8
10	27	♀	Busulfan	13.0	180	11.5	3	8
11	25	♂	Busulfan	9.3	532	232.0	36	2
12	31	♂	Busulfan	14 0	335	29 0	17	0
13	41	♀	Busulfan	15.0	391	27 4	16	0
14	32	♂	Busulfan	14 6	173	27 1	22	3
			Busulfan	6.5	13	7 0	66	62
15	25	♀	Busulfan	11 2	446	47.0	22	12
			Busulfan	7.2	26	12.5	76	74
16	21	♂	Busulfan	9 9	107	4.5	68	16
17	71	♂	Busulfan	10.5	539	48.2	40	24



Fig. 1 Chromosomes 9 and 22 in three patients with Ph positive CML showing a typical translocation t(9q+ 22q−) The normal chromosome pattern in a cell from a healthy person is included for comparison.

graphed in a fluorescence microscope and the negatives were analysed using a TV set for contrast enhancement (4 5). Only the metaphases which were well spread and had a distinct Q-banding were evaluated. In general the metaphases thus selected amounted to 30–50% of the photographed ones. The number of metaphases evaluated in the bone marrow as well as in the peripheral blood are shown in Table II.

RESULT

The Ph chromosome was found in 16 patients (9 of them have previously been reported in a preliminary communication (11)) (Table II). Chromosome 9 had additional chromosomal material on its long arm in 15 of these cases The amount of additional material appeared to be approximately the same as that lacking on chromosome 22 (Fig. 1)

Chromosome 9 was normal in one patient despite the presence of the Ph chromosome As described previously this patient had additional chromosomal material on chromosome 19 (12) suggesting a translocation between this chromosome and chromosome 22. The course of the disease appeared not to be different from that in other Ph positive patients and the survival was about three years (Table II).

One patient was Ph negative. Chromosome 9 was normal (Fig. 2) All other chromosomes also appeared to be normal in this patient.

Three patients (nos 14 15 16) with Ph positive CML were investigated in the blastic phase of the

Table II. *Chromosome abnormalities stage of disease and survival*

C=chronic, B=blastic

Pat. no.	Date of investigation	Stage of disease	No. of metaphases analysed: Bone marrow	Peripheral blood	Chromosomal abnormality	Survival from day of diagnosis
1	June 12 1972	C	10	6	9q+ 22q−	595*
2	April 24 1972	C	2	10	9q+ 22q−	746
3	April 17 1972	C	–	5	9q+ 22q−	752*
4	Oct. 1 1973	C	–	23	9q+ 22q−	220*
5	Sept. 25 1973	C	–	7	9q+ 22q−	229*
6	June 19 1973	C	14	–	9q+ 22q−	302*
7	April 24 1974	C	14	–	9q+ 22q−	10*
8	July 22 1974	C	12	–	9q+ 22q−	25
9	Dec. 12 1972	C	15	–	9q+ 22q−	1 494
10	Nov 17 1971	C	7	–	9q+ 22q−	1 556
11	Jan. 2 1973	C	14	–	9q+ 22q−	531
12	May 8 1972	C	8	–	9q+ 22q−	3 460*
13	Sept. 27 1974	C	13	17	19q+ 22q−	1 090
14	Dec. 20 1972	C	11	5	9q+ 22q−	243
	Jan. 24 1973	B	3	12	9q+; 22q− 7p− 8+ Ph +	
15	Nov 6 1973	B	20	2	9q+ 22q− 8+ 11+ 12+ 16+ 17+ 19+ Ph +	635
	Nov 29 1973	B	–	10	9q+ 22q− 7+ 8+ 11+ 17+ 19+ Ph+	
16	Aug. 24 1971	B	14	–	9q+ 22q−	575
17	May 14 1973	B	5	2	Normal	38

The patient is still alive.

disease. One of these patients (no. 14) was investigated both in the chronic and in the blastic phase.

The first investigation in patient 14 was made at a stage when a few blast cells were present in the peripheral blood but with no other hematological signs of a blastic phase. At this time the patient had the common pattern of a $t(9q+\ 22q-)$ abnormality in all metaphases investigated but in two of ten he had an additional Ph chromosome One month later the patient entered a blastic phase with 62% blast cells in the peripheral blood. At this time he had in addition to the previous abnormality ($t(9q+\ 22q-)$) an extra chromosome 8 and one chromosome with a shorter than normal short arm ($7p-$) in ten of twelve investigated metaphases in the peripheral blood, and in two of three investigated ones in the bone marrow All metaphases had now two apparently identical Ph chromosomes (Fig. 3)

Patient 15 was investigated twice in the blastic phase of the disease and had on both occasions several extra chromosomes (Table II) As patient 14 had two apparently identical Ph chromosomes in all metaphases analysed and an extra chromosome 8 in seven of thirteen metaphases in addition to the



Fig 2 Normal chromosomes 9 19 and 22 in patient with Ph negative CML (no 17). The pattern in Ph positive patient with the $t(9q+\ 22q-)$ pattern (no. 5) and in the patient (no. 13) with the $t(19q+\ 22q-)$ pattern are included for comparison.

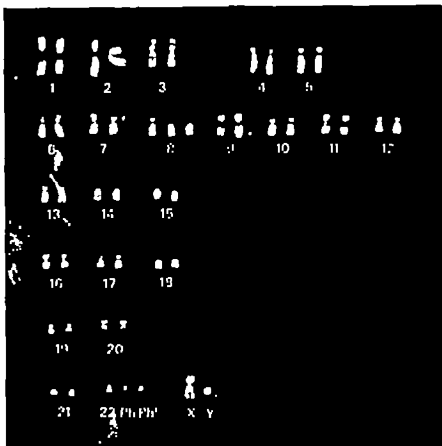


Fig 3 The karyotype of patient 14 with CML in the blastic phase showing the t(9q+ 22q−) pattern, an extra chromosome 8, a shorter than normal short arm on chromosome 7 (7p−) and two Ph' chromosomes.

t(9q+ 22q−) abnormality On the first occasion she had in addition an extra chromosome 19 in all of the eleven evaluable metaphases and also extra 11 12, 16 and 17 chromosomes in some of the cells. A similar aneuploid pattern was seen about three weeks later (Fig. 4).

The third of the patients in the blastic phase (no 16) had the t(9q+ 22q−) pattern and a normal number of chromosomes in the few metaphases investigated the number of which was however not sufficient to make sure that extra chromosomes were not present in some cells

One patient with CML in early blastic phase (no. 17) was Ph negative. Chromosome 9 as well as all other chromosomes were normal and the number of chromosomes was also normal in all metaphases investigated The hematological picture was typical of CML. The percentage of mature granulocytes in the peripheral blood was 60 and of myelocytes+ metamyelocytes 16 Furthermore the patient had thrombocytosis (539 000/mm³) and low alkaline phosphatase score as well as a decreased amount of PAS-reactive material in neutrophils indicative of CML (10) The number of colony-forming cells in the peripheral blood was characteristically increased in contrast to what is generally found in acute myeloblastic leukemia (14) The course of the disease was rapid and the patient succumbed in a sudden pulmonary oedema probably unrelated to the disease. At that time he had been treated with vincristine and prednisolone and had a hematological picture of an early blastic stage of CML (Hb 10.7 g/100 ml WBC 117 000/mm³ differential count bands 23.5% segmented neutrophils 13% eosinophils 0.5%, lymphocytes 10.5% monocytes 18.5% myeloblasts 6.5% promyelocytes 0.5%, myelocytes 20% metamyelocytes 7%).

DISCUSSION

It has recently been shown using the Q- and G-banding techniques that chromosome number 9 has additional chromosome material on its long arm in CML (20) This finding has repeatedly been con-

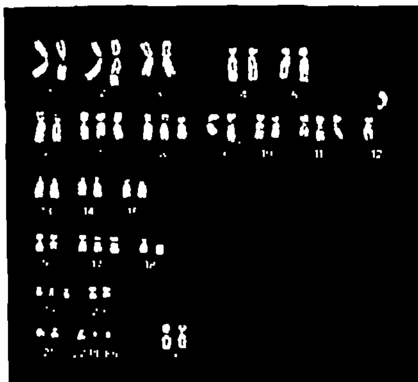


Fig 4 Patient 15 with Ph positive CML, $t(9q+\ 22q-)$, in the blastic phase showing extra chromosomes 7 8, 11 17 19 and two Ph chromosomes.

firmed (1 2, 11). Although it has not yet been quantitatively assessed it appears that the amount of additional material on chromosome 9 is in most patients about the same as that lacking on chromosome 22. It has therefore been suggested that there is a translocation between chromosomes 9 and 22 in CML (11 12, 20) However four patients, in whom chromosome 9 was normal despite the presence of the Ph chromosome have recently been described (9 12, 14 19). In one of these patients additional chromosome material was found on chromosome 2 (16) and in the others on the homologous chromosome 22 (9) and on chromosome 19 (12) respectively In the fourth of these patients it was not possible to find additional chromosome material on any other chromosome using the Giemsa technique (19) It was therefore suggested that this was a true deletion of chromosome 22 but it can not totally be excluded that small amounts of chromosome material had been translocated to a position where it is difficult to detect.

In the present study we report on one further CML patient with a normal chromosome 9 All other chromosomes in this patient were normal and there appeared to be no chromosomes with additional chromosome material In contrast to the previously described patients this patient was Ph negative. This observation gives further support to the view that there generally is a true translocation in Ph positive patients although it may involve not only chromosomes 22 and 9 but also chromosome 22 and other chromosomes Additional chromosome material on chromosome 9 in Ph negative patients would have indicated an aberration independent of the Ph chromosome This possibility can now be rejected and furthermore it appears that the presence of additional chromosome material on chromosome 9 or other autosomes is of less importance for the development of clinical CML, than the presence of the Ph In the course of our studies Rowley also found a normal karyotype in Ph negative CML (22) a further support to this view Non-detectable minute amounts of chromosome material might have been translocated in Ph negative patients, but it is also possible that the normal karyotype reflects on the possibility that Ph negative CML is a disease different from Ph positive

Chromosome aberrations in addition to the $t(9q+\ 22q-)$ pattern appear to be early sign of blastic transformation of CML. The most commonly observed aberration is the occurrence of extra chromosomes (22) and duplication of the Ph

chromosome (21) Extra C group chromosomes have been reported in several instances (13 15 23) and it is of particular interest that in the two patients with aneuploidy the extra chromosome was found to be number 8 Such a finding has previously been made in patients with sideroachrestic anemia and acute myeloblastic leukemia (7 8 17 18) In the course of our studies it was suggested that the extra C chromosomes in five CML patients in the blastic phase were identical with chromosome 8 (21) It thus appears that an extra chromosome 8 in hematopoietic cells is in some way associated with hematological malignancies particularly with those which have a potential for being transformed to a myeloblastic type of leukemia. In CML this chromosome appears late in the course of the disease and indicates the *terminal stage*

So far there are no data in the literature as to the frequency of the appearance of the other extra chromosomes noted in this investigation. The current use of the Q-banding and Giemsa-banding techniques may eventually show that a series of specific chromosomal abnormalities may develop during the course of blastic transformation of CML.

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IMMUNOGLOBULIN ANALYSIS IN FAMILIES OF MYELOMA PATIENTS

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Abstract The electrophoretic plasma protein pattern (in search for M-components) and the immunoglobulin (IgG IgA and IgM) levels have been studied in 24 members of two families with known familial occurrence of myeloma and in 124 relatives of ten patients with myeloma but without known familial occurrence of the disease Immunoglobulin levels were also determined in control material composed of 54 blood donors of both sexes. In one of the families with multiple occurrence of myeloma a mother and her son had the disease. In the other family two sibs had myeloma and one sib had an asymptomatic M-component. No further M-component was found among the 24 investigated relatives in these two families. In two of the other ten myeloma families an M-component was disclosed in an asymptomatic relative. The number of M-components found in the present study cannot be considered unduly high, as the prevalence of M-components in an adult population is about 1/100. The distribution of immunoglobulin levels among relatives differed little from that of controls and very few markedly deviating immunoglobulin levels were found. The study thus did not indicate a defective regulation of the immunoglobulin production within myeloma families Apparently myeloma differs in this respect from Waldenström s macroglobulinemia, which disease has been found preferentially to develop within families in which the macroglobulin (IgM) producing cell system is improperly regulated and therefore presumably predisposed to neoplastic transformation.

The possibility of a genetic predisposition to myeloma is suggested by the multiple occurrence of the disease within families (2, 5 7 9–11 19 21–25 28 32, 34) Waldenström s macroglobulinemia, the other major neoplastic plasma cell dyscrasia (26) also clusters within families (31). Furthermore a wide variety of immunoglobulin abnormalities has been found in asymptomatic relatives of patients with Waldenström s macroglobulinemia (15 31). Most frequent of these were polyclonal hypergammaglobulinemia and monoclonal hyper gammaglobulinemia, type IgM. This suggests the presence of an improperly regulated and/or hyper reactive IgM-producing cell system within these families Such a cell system might be more susceptible to oncogenic stimuli and the familial clustering of Waldenström s macroglobulinemia would thus be accounted for

Neoplastic plasma cell dyscrasias can be induced or occur spontaneously in certain strains of mice e.g. BALB/c mice which also respond with a stronger and more prolonged antibody response to a standard antigen challenge (26) These experimental results thus support the supposition that an improperly regulated and/or hyperreactive plasma cell system might predispose to the development of neoplastic plasma cell dyscrasias

Leukemic cells in chronic lymphocytic leukemia have been shown to carry immunoglobulins on their surface and they might consequently be considered as neoplastic B lymphocytes (1) and closely related to the neoplastic cells in myeloma and Waldenström s macroglobulinemia. Familial occurrence of chronic lymphocytic leukemia has long been recognized (29) and in some families immunoglobulin abnormalities have been demonstrated in asymptomatic relatives (8 27) indicating an inherited defect of B lymphocytes within these families

The present work is an attempt to elucidate whether familial aggregation of myeloma cases might be accounted for by an improperly regulated and/or hyperreactive plasma cell system within myeloma families. The electrophoretic plasma protein pattern (in search for M-components) and the immunoglobulin levels have been stud two families with known f occurr

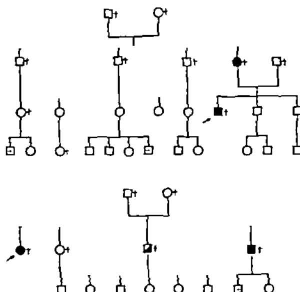


Fig. 1. Pedigrees of families I. 1 (top) and I. 2 (bottom) in material I. □=male ○=female, ↗=proband, ■●=myeloma, ◪◑=asymptomatic M-component, ⊡⊙=investigated relative †=dead.

myeloma and in the relatives of ten patients with myeloma but without known familial occurrence of the disease

MATERIALS

Material I consists of two families with known familial occurrence of myeloma attending the Department of Internal Medicine County Hospital of Skövde. In one family (I. 1) a mother and her son had myeloma. In the other family (I. 2) two sibs had myeloma and a third sib had an asymptomatic M-component. These two families have been previously reported (5). The pedigrees of the families are depicted in Fig. 1

The proband of family I. 1 had two M-components, IgGK and IgAK. His mother had a single M-component, type IgGK. Neither had Bence Jones proteinuria. The electrophoretic mobility of the M-components was dissimilar The son had an unequivocal myeloma. Even if the course of the mother illness was not quite typical of myeloma, this was considered to be the most adequate designation of her plasma cell dyscrasia. She had a rather large M-component (about 30 g/l), albeit stationary for more than 10 years. The proportion of plasma cells in the bone marrow aspirates was 15–20% and clusters of polymorphous and multinuclear plasma cells were also found. The skeleton showed general osteoporosis and collapsed vertebrae but no osteolytic lesions. She had a grave anemia and hepatosplenomegaly She died shortly afterwards when hospitalized for a traumatic fracture of her left collum femoris. No post mortem examination was performed. Sixteen relatives in family I. 1 were investigated. The relatives of the father could not be traced. The plasma protein pattern of eight relatives had been examined before the previous report and no abnormalities had been found. T o of these eight relatives later died. No post-mortem examination was performed but there were no indications of myeloma clinically

The proband of family I 2 had a myeloma with exclusively light chain myeloma protein, type lambda. One brother had a Bence Jones proteinuria, also type lambda, but of a different electrophoretic mobility At the time of the previous report he had shown no other clinical signs indicating myeloma. Some years afterwards, five years after the detection of the Bence Jones protein, the patient had multiple osteolytic lesions and about one-fifth of the nucleated cells in the bone marrow aspirate were polymorphous, flaming plasma cells with intranuclear inclusions. A prominent sharp band of light chains was at the same time seen in the serum electrophoresis. The kidney function rapidly deteriorated and he died in uremia. Another brother had an M-component, type IgGK, that remained stationary and no clinical signs indicating myeloma had appeared when he died, during an episode of hematuria and fever six years after the detection of the M-component. No post-mortem examination was performed. A fourth sib, a sister had died in cancer corporis uteri some years earlier The eight children of the sibs were all investigated (Fig. 1). Only few more distant relatives could be traced and plans for more extensive family study had to be abandoned.

In both the families I. 1 and I. 2 the cause of death of deceased relatives was inquired into The information gained was usually scarce but in no case were there any indications of myeloma.

Material II consists of the families of 10 myeloma patients attending the Department of Internal Medicine, University Hospital, Lund At the time of selection of probands there were 24 myeloma patients attending the department. They were all interviewed and informed about the study If they had a fair number of accessible relatives (6 or more) and if their attitude was positive, they were included in the study as probands

The probands are characterized in Table I. Nine had osteolytic lesions, an M-component either in serum or urine and a bone marrow picture indicative of myeloma. One proband had no osteolytic lesions but the bone marrow picture was unequivocal, with 50% plasma cells and a prominent M-component, type IgGL, (20 g/l) in serum. Relatives aged 15 years or less were excluded from the study as the immunoglobulin levels during childhood and early adolescence markedly differ from those of adults (33 36) which would hav complicated the interpretation of the immunoglobulin levels found during the study There then remained 130 living relatives, 58 of whom were first degree and 72 second degree relatives. All first degree and 66 second degree relatives have been investigated. Six relatives could not be persuaded to participate. There were no indications that deceased relatives had suffered from myeloma.

Controls An Australia antigen negative blood donor material composed of 34 adult individuals of both sexes was used as control material.

Table I *Clinical and laboratory data of the 10 probands in material II*

Proband no.	Sex	Age (y)	M-component: Immunological classification[a] Serum	Urine	M-component: Maximal concentration (approx.) (g/l) Serum	Urine	Plasma cells in bone marrow aspirates (%)	Radiological skeletal survey	Relatives (>15 y) First degree Alive	Studied	Second degree Alive	Studied
1	o	59	IgAL		20		36	Osteoporosis, collapsed vertebrae osteolytic lesions in skull	7	7	8	7
2	♀	55		L		3	61	Lytic lesions in humera, collapsed vertebrae	8	8	9	9
3	♂	63	IgDL IgGK	L	10 3	1	86	Multiple lytic lesions	6	6[b]	6	6
4	♀	54	IgGK		50		90	Multiple lytic lesions	4	4[b]	6	6
5	♀	62		L		14	17	Multiple lytic lesions	2	2	3	3
	o	64	IgGK	K	5	4	8	Multiple lytic lesions	3	3	3	1
	♂	70		K		6	93	Multiple lytic lesions	5	5	12	12
8	♂	75	IgGK		35		65	Multiple lytic lesions	10	10	12	11[c]
9	♀	62	IgGL		25		50	Osteoporosis, somewhat irregular	9	9	11	11
0	♂	57		K		3	7	Multiple lytic lesions	4	4	2	0
									58	58	72	66

K=kappa, L=lambda light chains.
In one relative only agarose electrophoresis performed.
M-component found in one relative.

METHODS

A venous sample (10–20 ml) was drawn either in the laboratory or by local nurse, in which case it was sent by post to the laboratory. Serum was separated within 24–48 hours and stored at −20°C until analysed.

All sera were screened for M-components with agarose electrophoresis (13). When sharp bands, suspected to be M-components, were observed, immunoelectrophoresis according to Scheidegger (30) was performed. Specific antisera to heavy chains (γ, α and μ) and to light chains (and λ) were then used. N quantitative determination of M-components was performed. The concentration of the M-component was, however estimated by visual inspection of the M-component zone in relation to the albumin and the transferrin zones in the agarose electrophoresis. When an M-component was found by serum agarose electrophoresis, urine was also submitted to electrophoresis after concentration. Quantitative determination of IgG IgA and IgM was performed on all sera except on one, which was spoiled by accident after the performance of agarose electrophoresis. IgG and IgA levels were determined by electroimmunoassay (18). IgM levels were determined by the single radial immunodiffusion method (20). The immunoglobulin levels in the present study are expressed in g/l. One mg immunoglobulin in our reference sera for IgG, IgA and IgM respectively corresponds to 11.5 (IgG) 59.5 (IgA) and 115 (IgM) potency units of the World Health Organization Serum Immunoglobulin Reference Standard (38)

RESULTS

M-components

No further M-component was found among the 24 investigated relatives in the two families constituting material I (the Skövde families).

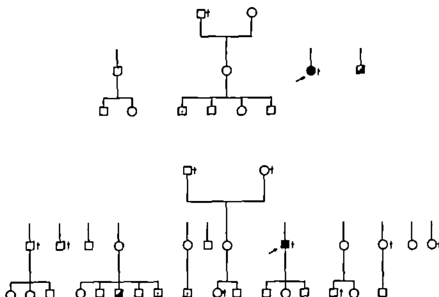


Fig. 2. Pedigrees of families II 4 (top) and II 8 (bottom) in material II Symbols as in Fig. 1

In material II an M-component was found in two of the ten investigated families The pedigrees of these two families are depicted in Fig. 2 The proband of family II 4 had a prominent IgGK M component. One of her three sibs a 50-year-old brother had an IgGK M-component, about 10 g/l of slightly different mobility (Fig. 3) The concentration of the M-component has been stationary ng a follow up period of two years Urine rophoresis has disclosed no Bence Jones ein. Repeated bone marrow aspirates have een essentially normal (maximally 5% plasma cells with no signs of immaturity) Thorough X-ray examination of the skeleton has been normal He has no anemia and is feeling well

The proband of family II 8 had also an IgGK M-component (Table I) A 60-year-old nephew had an IgGK M-component of a quite different mobility (the proband had an M-component with cathodal and his nephew an M-component with anodal mobility) The M-component was of low concentration about 5 g/l or less, and has been stationary during a follow-up of two years He has no Bence Jones proteinuria. There is no anemia and repeated bone marrow aspirates have been essentially normal (maximally 3% plasma cells with no signs of immaturity). Thorough X-ray examination of the skeleton has disclosed no lesions indicating myeloma.

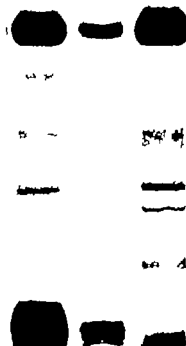


Fig. 3 Agarose gel electrophoresis of serum of the proband of family II 4 (left and middle) and of plasma of her brother (right). The serum sample in the middle was diluted tenfold before application.

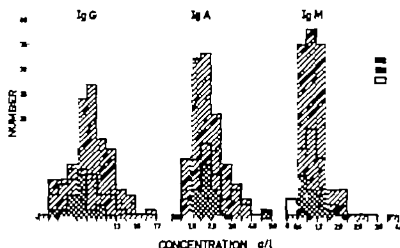


Fig. 4. Distribution of immunoglobulin levels among relatives in materials I (*M I*) and II (*M II*) and among controls (*C*).

Immunoglobulin levels

The distribution of immunoglobulin levels among relatives and controls is depicted in Fig. 4

The distribution of *IgG levels* in material I was very similar to that of controls whereas it possibly differed somewhat in material II from that of controls, the relatives IgG levels tending to be slightly higher No markedly increased or decreased levels were found however The distribution of *IgA levels* in both materials was similar to that of controls except for a few rather high IgA levels in material II. The distribution of *IgM levels* in both materials differed little from that of controls For unknown reasons however some remarkably high values were obtained in both materials as well as in controls

DISCUSSION

The prevalence of M-components in an adult population is about 1/100 (3) All but a minority are asymptomatic (4) In the present study two M components both asymptomatic were disclosed among 124 relatives of 10 myeloma patients in material II. This number is consequently quite in accordance with the known prevalence figure In material I, the Skövde families one asymptomatic M-component was already known at the time of the study but no further M-components were found among 24 investigated relatives, so the number of asymptomatic M-components in these two families cannot be considered unduly high

Immunoglobulin levels vary with age, sex, race and environmental influences (6 12 14 33 36) and such variations should be taken into account when control materials are selected The blood donors used as controls in the present study were adult individuals of both sexes but differed somewhat from the myeloma material in age distribution (many relatives in the myeloma families being quite elderly) sex distribution (within different age groups) and probably environmental influences (although most controls and most relatives lived in rural areas or in small towns) The controls, however represented a fair average of a healthy adult population which might be considered sufficient for the purpose of the present investigation i.e to elucidate whether the immunoglobulin levels in relatives of myeloma patients indicated an improperly regulated and/or hyperreactive plasma cell system more liable to neoplastic transformation than the plasma cell system of other individuals. If this was the case, one could reasonably expect the immunoglobulin levels in the myeloma families to show a decisive deviation from the average The distribution of immunoglobulin levels in relatives and controls in the present study was, however rather similar Very few relatives had markedly deviating immunoglobulin levels

Two other systematic surveys of myeloma families have been published both of which also included probands with M-components from other causes In the survey of Williams et al (37) two M-components both asymptomatic were disclosed among 205 relatives of 17 myeloma patients This number of M-components was thus quite in ac

cordance with the known prevalence figure of 1%. In the survey of Spengler et al. (32) the prevalence of M-components was about the same with three M-components disclosed among relatives of 14 myeloma patients One of these M components was associated with a myeloma, the others were asymptomatic In both surveys the information on the individual immunoglobulin levels of relatives is scarce. Spengler et al refrained from carrying out quantitative determinations of individual immunoglobulins as most of the serum electrophoretic and immunoelectrophoretic patterns were normal or approximately normal. Williams et al performed quantitative estimation of IgG IgA and IgM on all sera. They report on one myeloma family in which three relatives had high polyclonal IgG levels They did not comment on the immunoglobulin levels of the other relatives except for the statement that they had found a close quantitative relationsship to IgG IgA and IgM within many sibling groups of the kindreds studied.

The available information from family surveys of myeloma probands including ours thus does not indicate any unduly high frequency of M components or immunoglobulin abnormalities in myeloma families. Accordingly there is no support for the suggestion of the existence of an improperly regulated and/or hyperactive plasma cell system n myeloma families predisposing to the se The possibility can, however not be ex ed that a familial predisposition to myeloma xists, which is in fact suggested by a few quite impressive reports on familial myeloma. Thus in a Dutch family two sibs had myeloma and one sib had an asymptomatic M-component. One of the myeloma sibs had one child with myeloma and two children with asymptomatic M-components (24). The immunoglobulin levels in the remaining relatives were essentially normal. In another report two sibs had, and the father might have had myeloma (25). In a third report three sibs had myeloma (2) Most other reported familial myeloma cases are, however the incidental discovery of two family members afflicted with the disease such as our Skövde cases (7 9–11 19 21–23 28 34) Such an observation might be accounted for by chance, as myeloma is not an altogether rare disease the incidence being about 3/100 000 a year (35). In fact, most myeloma cases are non-familial. Thus among 140 myeloma cases seen by Waldenström (35) there were no siblings and of about 550 myeloma cases seen at the Mayo Clinic (10) only two were siblings. The two family surveys referred to earlier indicated only one additional myeloma case among 31 investigated families (32, 37) this being a second degree relative. Nor did our survey disclose any further myeloma cases.

With the exception of a few impressive familial clusterings of myeloma there is thus not much indication of a familial predisposition to the disease. Familial clustering of myeloma, if not fortuitous, might be due as much to common environmental influences as genetically determined. The occurrence of myeloma within four pairs of spouses (16) and a reported geographic clustering of the disease (17) suggest that this could be the case

ACKNOWLEDGEMENT

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ERYTHROBLASTIC ISLANDS AND INEFFECTIVE ERYTHROPOIESIS IN VITAMIN B_{12} DEFICIENCY

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Abstract. Bone marrow smears from 4 patients with anemia due to vitamin B_{12} deficiency have been compared with smears from healthy controls. A three fold increase of erythroblastic islands and predominance of basophilic erythroblasts were found in the patient group. There was firm, positive correlation between the proportions of basophilic erythroblasts within the erythropoietic pool and the numbers of erythroblastic islands. It is suggested that the large numbers of erythroblastic islands in the patient group are a morphological sign of the ineffective erythropoiesis known to characterize megaloblastic anaemia. The results are compatible with an intramedullary death of especially polychromatic erythroblasts and phagocytosis of these cells by reticulum cells.

Using different techniques, several investigators have presented data that convincingly point to an intramedullary destruction of erythroid precursor cells as an important feature of the ineffective erythropoiesis in megaloblastic anaemia (5 8 10 11) From an electron microscopy study t has been concluded that phagocytic reticuloendothelial cells of the bone marrow may devour erythroblasts in patients with pernicious anaemia (6)

The present investigation was undertaken to find out whether there are any consistent morphological signs in the bone marrow of patients with megaloblastic anaemia pointing to increased erythroblast phagocytosis Special interest was then devoted to the presence in the bone marrow of reticulum cells containing cell debris and in contact with erythroblasts "erythroblastic islands" since previous results have suggested that an abundance of such formations may indicate an active phagocytosis of red cell precursors by the reticulum cells which is compatible with an ineffective erythropoiesis (4 12).

MATERIAL AND METHODS

Patients Twenty-four patients, 13 women and 11 men, aged 46–87 years (mean 68) ith anaemia due t vitamin B_{12} deficiency were xamined Hb 5 0–10.5 g/100 ml (mean 7.2), serum B_{12} 5–90 pg/ml (mean 36), serum iron 11 ?90 μg/100 ml (mean 195) TIBC 207–320 μg/100 ml (mean 269) MCV 111–140 μ^3 (mean 123). Of the erythroid precursors 73–98% (mean 87) were megaloblasts.

Controls Eleven apparently healthy persons 6 women and 5 men, aged 41–82 years (mean 64) served as controls. Hb 12.0–16.5 g/100 ml (mean 14 2) all had normal WBC and normal differential counts ESR was normal in all and no megaloblasts or other abnormalities were found in their bone marrow

Examination of bone marrow smears Bone marrow was obtained through conventional sternal puncture and smears were stained with May-Grünwald-Giemsa. 1 000–3 000 (mean 1 160) erythroblasts were classified according to the stage of maturation. Proerythroblasts and basophilic erythroblasts were then pooled into one group designated basophilic erythroblasts A mitotic index of the erythroblasts was determined and the number of erythroblastic islands per 1 000 erythroblasts was recorded

RESULTS

The mitotic indices of the erythroblasts were largely the same in the patients and the controls on an average 36 and 31/1 000 respecti ely (Fig. 1) The composition of the erythroid precursor pool with regard to different stages of maturation differed between patients and controls. Thus the proportion of basophilic erythroblasts was on an average 55% in the patients and 31% in the controls ($p<0.001$) (Fig. 2).

The average number of erythroblastic islands was 18.2/1 000 erythroblasts i the patient group and only 5 8 in the healthy control (Figs. 3 and 4) The difference is significant ($p<0.001$)

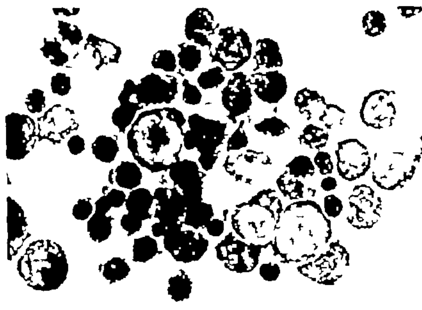


Fig. 4. Reticulum cell surrounded by megaloblasts in a patient with vitamin B_{12} deficiency.

effective erythropoiesis. It has been claimed that the presence of erythroblastic islands in the bone marrow indicates a nursing process in which erythroblasts obtain ferritin from the reticulum cells (3), but it has also been suggested that such formations may indicate an intramedullary phagocytosis and destruction of erythroid cells by the reticulum cells and thus be an expression of ineffective erythropoiesis (4, 12). With regard to megaloblastic anaemia the latter interpretation seems to be the most likely since, as mentioned, intramedullary death and phagocytosis of erythroblasts are typical of this condition.

A firm correlation between the proportions of basophilic erythroblasts within the erythropoietic pool and the frequency of erythroblastic islands in the bone marrow was found in the present investigation. In the patients with the largest proportions of basophilic erythroblasts the erythroblastic islands were also most abundant. As mentioned, polychromatic erythroblasts have a high death rate in megaloblastic anaemia, causing a predominance of basophilic erythroblasts. It is highly probable that the dead erythroblasts are eliminated through phagocytosis by reticuloendothelial cells. Therefore the presence of large numbers of reticulum cells containing cell debris and in close contact with erythroblasts may be the morphological sign of a high death rate among erythroid precursors.

Apart from the megaloblastic changes, a very similar pattern showing increased proportions of basophilic erythroblasts combined with increased numbers of erythroblastic islands has previously been found in patients with anaemia associated with inflammatory lesions or malignant disease (4, 17). It is therefore probable that intramedullary death and phagocytosis of erythroblasts may contribute to anaemia also in such patients and that

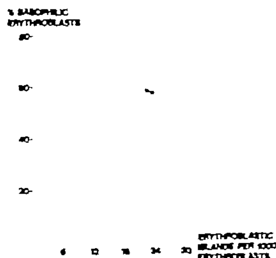


Fig. 5. Relation between the numbers of erythroblastic islands/1 000 erythroblasts and the proportions of basophilic erythroblasts within the ... pool.

high numbers of erythroblastic islands in anaemic patients are a sign of ineffective erythropoiesis irrespective of the aetiology of the condition.

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COAGULATION STUDIES IN HEMOLYTIC UREMIC SYNDROME AND THROMBOTIC THROMBOCYTOPENIC PURPURA

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Abstract. Seven patients with hemolytic uremic syndrome (HUS) and 4 with thrombotic thrombocytopenic purpura (TTP) have been studied with almost daily determination of urinary fibrin/fibrinogen degradation products (FDP) and the coagulation and fibrinolytic parameters in blood during the course of the disease. These studies have shown that intravascular coagulation might occur not only in TTP but even in an early phase of HUS. In all these cases there was a good correlation between the clinical course and the increase in platelet count and disappearance of FDP in the urine. The best way of handling the patients with HUS was an early institution of hemodialysis in combination with low dosage heparin. In the patients with TTP the best results were obtained with hemodialysis and adequate continuous heparin treatment.

Disseminated intravascular coagulation or generalized defibrination has been reported to occur in hemolytic uremic syndrome (HUS) and in thrombotic thrombocytopenic purpura (TTP) (1 10 4 31). But most coagulation studies of these syndromes have not revealed any signs of consumption coagulopathy or increased fibrinolysis (9 18 23 28 34). The efficacy of treatment with heparin has therefore been questioned (16 23 36). Treatment of the acute renal failure with dialysis seems to be of utmost importance in these patients (17 36).

In 7 patients with HUS and 4 with TTP coagulation and fibrinolysis were studied in detail to find out whether these syndromes include intravascular coagulation.

METHODS

The following determinations were made of the platelets, coagulation factors and components of the fibrinolytic system: platelet count, bleeding time (Duke), recalcification time of plasma, one-stage prothrombin time, prothrombin+factor VII factor X (Owren's P&P test), factor V, factor VIII (biological activity and immunochemical determination), fibrinogen, fibrinolytic activity of plasma and resuspended euglobulin precipitate on fibrin plates, plasminogen (immunochemical method), fibrin/fibrinogen degradation products (FDP) in serum and urine, and ethanol gelation test. Unless otherwise stated, the methods described earlier were used (8 14 30).

Platelet counts were made by the method of Björkman (2).

Factor VIII was immunochemically determined according to Holmberg and Nilsson (15).

Ethanol gelation test was performed according to Godal et al (1).

FDP in serum and urine were determined by the immunochemical method of Nilehn (29) with specific antiserum against the D-product using the rocket method of Laurell (22). The serum or urine samples (dil 1/1) were applied to agarose plates containing the anti-D serum. On high voltage electrophoresis the antigen is forced into the gel. Precipitation peaks form and their heights are proportional to the amount of antigen in the sample. High molecular weight degradation products were used as standard (13).

Platelet survival studies were performed in the way described by Ljungqvist and Bergentz (26). The ^{51}Cr activity in platelet-rich plasma was measured repeatedly for 48 hours after infusion of ^{51}Cr-labeled normal platelets.

Fibrinolytic activity of the vessel wall was studied by the immunochemical method described by Pandolfi (31).

Dialysis treatments. The children were given treatments with disposable plate kidneys of total surface of 0.3 or 0.16 m². The blood tubing and dialyser were primed with blood (about 120 ml), to which 1000 U heparin/1 000 ml had been added. For the adult hollow fiber kidney was used as dialyser. A dialysate flow of 1 l/min, blood flow of 100–200 ml/min and ultrafiltration of 60–700 mmHg were used.

Cannulation. The silastic-teflon arteriovenous cannula system described by Quinton et al. (33) was placed in the radial artery and in the cephalic vein. In one patient the brachial artery was cannulated.

Parenteral nutrition. During the acute stage of the disease the patients were nourished with essential amino

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9	10	11	
♂	♀	♀	Normal
31	49	25	range
10 0	13.0	0.9	0.9–1.2
290	290	290	
4 000	42 000	64 000	200–400 000
10	0	16	40–175
213	208	370	60–160
		–	60–175
90	128	28	80–120
63	100	32	80–120
0 62	0.32	0.20	0 20–0 40
25	30	750	0–5
	70	50	0
	Pos.	–	Neg.
96	120	15	70–130
	Steroid	HD	
HUS Malign. hypert.	HUS SLE	Postpart. ren fail. Defibr	

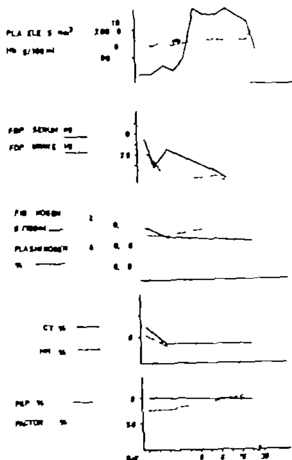


Fig 1 Case 1

ment with hemodialysis 4 times. After 3 days the production of urine started and renal function successively became normal.

Case 3 (Fig 3)

A 1-year-old girl. Four to five days before admission to hospital she vomited and had diarrhea. Disseminated petechiae. No urinary production. Hb 7.8 g/100 ml, platelets 26 000/µl, reticulocytes 74 000/µl, serum creatinine 3.. mg/100 ml. During the first few days the patient had repeated attacks of convulsions. Treatment with hemodialysis was started. To prevent clotting in the shunt, heparin was given (250 IU×4) from the 3rd to the 9th day as well as about 1 500 IU during treatment with hemodialysis 6 times, about 4 hours each time. The patient's condition was extremely poor and she had repeated attacks of convulsions, but after 10 days the urinary production increased, dialysis was stopped, and the patient recovered.

Case 4 (Fig 4)

A girl, aged 2 ½ years, was admitted to hospital after 6 days diarrhea and vomiting and no production of urine during the last 3 days. Physical examination pale apathetic girl in poor condition with repeated seizures. Hb 5.9 g/100 ml, platelets 42 000/µl and serum creatinine 10 mg/100 ml. Th blood film revealed Burr cells. During the first days the patient required peritoneal dialysis, and on the 3rd day hemodialysis was started, which was given also on the 4th and 6th day. Because of clotting in the shunt low dosage heparin (1 000 IU/day) was given besides the heparin administered during the dialysis (2 000 IU during the 5-hour dialysis). On the 6th day of treatment the rinary production increased and dialysis was no longer necessary. The patient recovered.

Case 5

A girl, aged 3 years, had always felt well until 1 week before admission, when she developed fever with vomiting, abdominal pain and diarrhea. On admission disseminated petechiae and ecchymoses. Hb 5 g/100 ml, platelets 6000/µl, reticulocytes 6..000/µl. Increased number of megacaryocytes in the bone marrow. Haptoglobin 6 mg/100 ml. Proteinuria and hematuria but serum urea and creatinine were within normal limits. Studies of the erythrocyte survival with [51]Cr-labeling revealed pronounced hemolysis. The patient was given large doses of steroids with no effect. Splenectomy was performed and azathioprine was administered. The pa

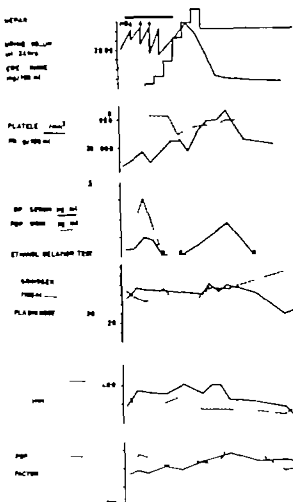


Fig 2 Case

tient wa given large amounts of fresh blood Neither the Hb nor the platelet count increased The patient s condition had until then been fairly good But renal function was now impaired and the BP rose Paresis of the left arm and leg appeared and she had repeated seizures and died Autopsy showed severe changes in the vessels of precapillary caliber in the form of endothelial hyperplasia, fibrinoid necrosis and microthrombi i most of the organs

Group II (cases 6–11) consi ted of 4 women and 2 men. Two women and two men with malignant hyper tension and involvement of multiple organs before ad mission. Their renal function deteriorated when the disease was complicated by development of TTP One woman who had had SLE for many years and one woman with postpartum renal failure developed HUS Two of the patients with malignant hypertension (nos. 6 and 7) and the woman with postpartum renal failure were given hemodialysis treatment during which they were heparinized. In these patients heparin was administered also between the dialyses intermittently or as a continuous infusion.

Case 6 (Fig 5)

A woman, aged 48 had always felt well until the end of 1971 when progressive occipital headache dizziness and blurred vision occurred. On admission petechiae on the legs and peripheral edema were seen. Papilledema, hemorrhages and exudates were demonstrated in her fundi. BP 250/110 mmHg, Hb 9 7 g/100 ml, platelets 20 000/µl haptoglobin 7 mg/100 ml. The blood film revealed anisocytosis, poikilocytosis and fragmented red cells. Serum bilirubin 1.6 mg/100 ml serum creatinine .. mg/100 ml. Platelet survival studies with ^{51}Cr labeled normal platelets showed a half-life of about 30 min. Fluctuating neurological signs and paresis of the left arm and leg developed. Hemodialysis was started because of oliguria with grave electrolyte deficits and pulmonary edema Continuous heparin infusion with 20 000 IU/day was started and the platelet count rose to 110 000/µl but decreased to 15 000/µl on the following days when heparin was withdrawn. The platelet count returned to normal and the coagulation factors improved during heparin infusion, which was continued for 5 weeks. After 7 treatments with hemodialysis the urine output increased but the serum creatinine persisted at a level of 5–6 mg/100 ml. The hyper tension was caused by a stenosis of the right renal artery Renal biopsy of the left kidney showed severe arteriolar sclerosis. After nephrectomy of that kidney

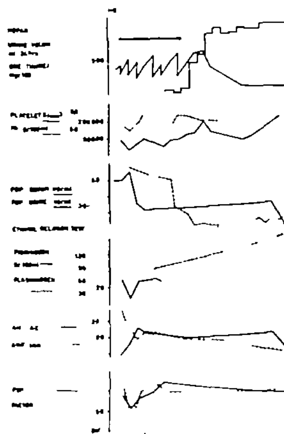


Fig 3 Case 3

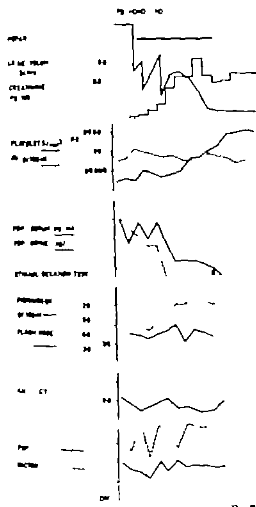


Fig 4 Case 4

and reconstruction of the renal artery stenosis, the renal function and BP became normal.

Case 7 (Fig 6)

A woman, aged 31 was admitted to hospital after 3 weeks of abdominal pain and vomiting. Laparotomy revealed gangrene of the small bowel caused by multiple thrombi in the mesenteric vessels. Postoperatively thrombus developed in vv portae and multiple thrombi in the arms and legs. Hb 5 g/100 ml platelets 4 000/µl haptoglobin 0 BP 250/140 mmHg. Examination of the ocular fundi revealed hemorrhages, exudates and papill edema. Serum creatinine 4 6 mg/100 ml Hemodialysis was started because of oliguria and decreasing renal function. The patient was treated 13 times with hemodialysis, about 6 hours on each occasion, during which 15-20 000 IU heparin was given. Heparin was administered subcutaneously 12 500 IU twice daily The platelet count rose and the renal function and various coagulation factors became normal during the treatment. After some months, however the patient died from abdominal and pulmonary abscesses.

Case 8

A 53-year-old man wa admitted t hospital because of severe occipital headache during the previous few weeks. On admission BP was 190/130 mmHg, the heart size as increased and ocular fundi showed exudates and hemorrhages. Hb 10.0 g/100 ml platelet 54 000/µl, reticulocytes L4 000/µl, haptoglobin 0 Serum creatinine was 3 0 mg/100 ml and rapidly increasing because of oliguria. During the following week multiple thrombi developed in the legs and cerebrum and the patient died Autopsy revealed severe arterios lerotic changes the vessels with multiple thrombi.

Case 9

A 51-year-old man with hypertension know for 2 years with cardiomegaly and changes in the fundi corresponding to KW III During this time serum creatinine had been stable at about mg/100 ml H was admitted t hospital because of deterioration. Hb 9 7 g/100 ml platelets 4 000/µl, haptoglobin 0 BP 40/140 mmHg and serum creatinine 10 mg/100 ml The patient be ame oliguric and died from pulmonary edema

Case 10

A woman aged 49 with known SLE with in ol of multiple organs since 1954 Until admission the rum creatinine level had been about mg/100 ml

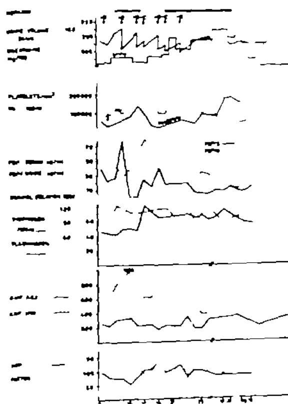


Fig 5 Case 6

admission multiple petechiae and ecchymoses. Hb 9.8 g/100 ml platelets 4 000/µl reticulocytes 778 000/µl and haptoglobin 0, serum creatinine 13 mg/100 ml The patient became oliguric and was treated with peritoneal dialysis but died.

Case 11 (Fig 7)

A 25-year-old pregnant woman who had felt well until 1 month before calculated delivery when she became icteric Serum bilirubin 8 mg/100 ml the transaminase values were high and the alkaline phosphatases considerably increased Caesarean section was done because of the impaired liver function. Examination of the various coagulation factors revealed a massive consumption Hb 9 g/100 ml platelets 64 000/µl haptoglobin 16 mg/100 ml and serum creatinine 3 7 mg/100 ml The patient received 20 000 IU heparin on the first day and 30 000 IU on the second day after operation as a continuous infusion, besides multiple transfusions of fresh blood Oliguria-anuria ensued. The

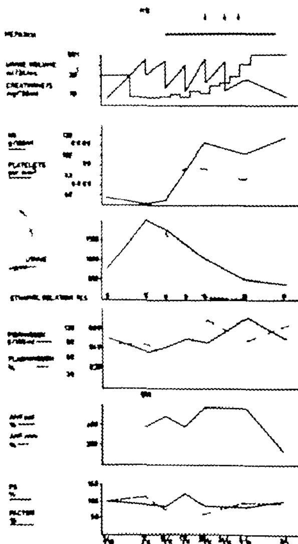


Fig 6 Case 7

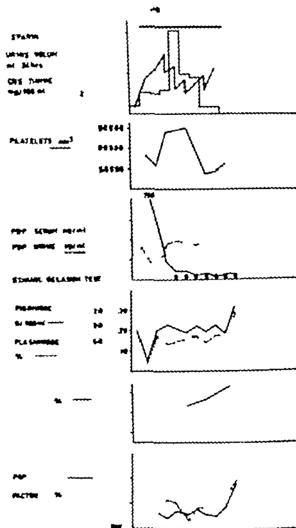


Fig 7 Case 11

heparin supply was continued and treatment with hemodialysis was started during the following day She was treated 4 times. During treatment the fibrinogen level rose and the platelet count and the coagulation factors became normal However the patient died in acute liver necrosis.

RESULTS

The results of coagulation analyses and some other relevant data on admission are summarized in Table I

All the 11 patients had low platelet counts hemolysis and renal damage of varying severity The initial levels of factor VIII as determined by the biological method were high in all patients except one Factor VIII determined immunologically in 4 patients was extremely high in all. Ten patients

ere examined for FDP which was invariably found in large amounts both in serum and urine On admission only one patient (no. 11) with post partum renal failure showed multiple defects with low levels of P&P factor V fibrinogen and plasminogen. Of the remaining 10 patients 8 had normal and 2 high P&P values factor V was normal in 7 and markedly increased in 3 The fibrinogen levels were either normal or high. The plasminogen level was normal

None of the patients showed increased fibrinolytic activity in the circulating blood assayed on fibrin plates The ethanol gelation test performed in the initial stage in five patients, was positive in only one (no 10).

All patients in group I had symptoms of HUS which were kept under observation by daily determinations of the various coagulation and fibrinolytic components (Figs. 1–4). Patient 1 received no heparin and hemodialysis was not performed As is seen from Fig. 1 the platelet count increased to normal and the FDP disappeared within one week which coincided with clinical recovery Patients 2, 3 and 4 were treated almost daily with hemodialysis. Heparin in very small doses was given for a short time to prevent clotting in the shunts The platelet counts became normal and the FDP in the urine disappeared. Only in case 3 (Fig. 3) could a marked decrease in the various coagulation factors be demonstrated on the second day after admission. On the following day the values again began to increase In all these cases there was a good correlation between the clinical course and the increase in platelet count and disappearance of FDP in the urine

Case 5 presented the clinical symptoms of HUS already on admission. Throughout the disease the fibrinogen level was normal P&P factor V and factor VIII were increased and the platelet count was low FDP were present in serum but were not determined in urine. She received no heparin or hemodialysis treatment. TTP developed with multiple thrombi in all organs and the patient died.

In group II the patients had complicated latent diseases. In two patients with malignant hypertension and TTP (nos 6 and 7) platelet survival studies and almost daily coagulation studies were performed (Figs 5 and 6). Both patients were found to have a rapid consumption of platelets the half-life being only 1/2–1 hour Initially both had normal fibrinogen values but high FDP levels both in serum and urine Factor VIII was also markedly increased while the other coagulation factors were largely normal The patients were treated with heparin and hemodialysis The course in case 6 is shown in Fig. 5 During treatment the platelet count and the fibrinogen increased while the concentration of FDP decreased A similar course was seen in case 7 (Fig 6) In both cases renal function became normal

In cases 8 and 9 with malignant hypertension and in case 10 with SLE we followed only the platelet count. These patients received no hemodialysis or heparin treatment The platelet count remained low throughout the disease The level of the various coagulation parameters found on admission did not differ from those in the other patients in this group All these patients died in uremia. Patient 11 (Fig. 7) initially showed multiple coagulation defects She was treated with heparin and dialysis and the platelet count coagulation factors became normal but the disease progressed and the patient died in liver necrosis and uremia.

DISCUSSION

There is no agreement on the role played by disseminated intravascular coagulation in the pathogenesis and symptomatology of HUS and TTP Anticoagulation with heparin has been employed with varying success in patients with this syndrome (10 36) In most reported cases no detailed studies of the coagulation and fibrinolytic factors have been performed throughout the disease though such studies are important for evaluating the role played by intravascular coagulation and anticoagulation treatment in these syndromes

The five patients our group I fulfilled the criteria of HUS All of them were followed by daily determination of the various coagulation and fibrinolytic factors They had normal or raised levels of factors V and VIII P&P and fibrinogen. FDP in serum and in urine were demonstrated in high concentrations but no other signs of increased fibrinolysis in the circulation These results are in agreement with a local coagulation with fibrin deposits in the kidneys rather than intravascular coagulation, as was also found by Katz et al (18) and Lieberman (23). In case 3 however consumption of the coagulation factors was demonstrated the platelet nt the vels

of fibrinogen, factor V factor VIII and P&P de creased significantly but fibrinolysis was not increased The patient s condition deteriorated with fluctuating neurological symptoms and seizures which disappeared when coagulation factors and platelet count improved during continued treat ment (Fig. 3). The occurrence of generalized de fibrination can apparently occur in an early phase of these syndromes. This has also been suggested in earlier publications but detailed sequential studies of the coagulation factors were not per formed (1 10).

TTP has been described in combination with malignant hypertension (5 7 19 25 35) The severe arteriolar damage occurring in malignant hyp rtension and even in collagen diseases such as SLE expressed as hyperplasia of the intima and proliferation of the endothelial cells and fibrinoid necroses might produce abnormalities in the microcirculation, with the development of dis seminated intravascular coagulation and microangiopathic hemolytic anemia as a result (19) Similar vessel changes have been described in pre-eclampsia and postpartum renal failure (19)

Four of our patients in group II had malignant hypertension At least three of them developed TTP aggr ating their hypertension and renal damage They had a rapid consumption of plate lets. Th fibrinogen level was normal in spite of the h ghly act e disease indicating an increased ... ption Two were treated with heparin, w followed by normalization of the plate count ncrease in fibrinogen, disappearance of FDP and improvement of renal function (Figs 5 and 6).

In case 11 the FDP le el was very high and fibrin monomers could be demonstrated in the blood. The low oagulation factor levels might be due to a deficient synthesis because of the liver damage or indicate an activation of the coagulation process The high FDP level in serum is remarkable A essel biopsy examined by the histochemical method of Pandolfi (31) showed no occurrence of fibrinolytic acti ators in the vessel wall indicating that the patient had emptied most of the fibrinolytic activators from the essel walls to resolve the fibrin deposits occurring in most of the organs. Treatment with heparin in this case successively normalized the coagulation factors.

The pathogeneses of HUS and TTP are probably identical and only represent various degrees of manifestation of the same disease. In HUS there are usually only symptoms of thrombi and fibrin deposits from the kidneys resulting in various degrees of renal damage The syndrome can, however result in involvement of several organs, indicating occurrence of TTP with disseminated thrombi of the microcirculation. In case 5 it was apparent that the HUS might proceed to a generalized involvement of multiple organs which was verified at autopsy

In most cases of HUS autopsies show intrarenal thrombosis while TTP is accompanied by severe arteriolar disease and disseminated thrombi of fibrin in most organs (20)

As a rule no consumption coagulopathy has been demonstrated in the syndromes discussed, except rapid consumption of platelets (3 4 9 ...). Detailed coagulation studies in our patients have, however shown that intravascular coagulation occurs not only in TTP but also in an early phase of HUS as was also shown by Corrigan et al. (6)

Opinions differ on the effect of heparin in HUS. According to some authors (10 1 ..3) treatment with heparin can reduce the mortality rate among children with this syndrome while others have found no such effects of heparin (11 27 36)

Case 1 had a very mild form of HUS which did not require treatment and healed spontaneously Case 5 was treated neither with dialysis nor heparin the disease developed into TTP and the patient died from severe neurological and renal complications. The other three patients in our group I with HUS were given treatment with low dosage heparin in order to prevent clotting in the external shunts The quantity of heparin given in these cases was so low that it had no prolonging effect on the coagulation times The possibility of heparin preventing a continued activation of the coagulation process can however not be excluded The low dosage heparin might here act in the same way as in the prevention of complications with postoperative thrombosis by preventing the activation of factor X

Early institution of hemodialysis is probably of utmost importance in severe cases of HUS When our patients recovered their coagulation status became normal Accordingly in our experience the best way of handling patients with HUS is an early institution of hemodialysis in combination with low dosage heparin. In this way the uremic

symptoms and the nutritional problems of the patient can be handled in an effective way and a continued activation of the coagulation process can probably be prevented. If the disease proceeds, with the appearance of neurological symptoms or involvement of multiple organs adequate heparinization even between treatments with hemodialysis should be instituted promptly.

ACKNOWLEDGEMENTS

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SUCCESSFUL IMMUNOSUPPRESSIVE TREATMENT OF OLIGURIC EXTRACAPILLARY GLOMERULONEPHRITIS

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Abstract Ten consecutive patients with oliguric extracapillary glomerulonephritis have been treated with prednisone and/or azathioprine. Six patients responded favourably to the treatment, while 4 died of uraemia. Of the 6 responders 5 were treated with prednisone and azathioprine and one with prednisone. The 6 patients are still on immunosuppressive treatment and have creatinine clearances of 1–75 ml/min 2–43 months after start of treatment. Thus treatment with prednisone and azathioprine seems to improve the prognosis of oliguric extracapillary glomerulonephritis.

The clinical course of extracapillary glomerulonephritis is that of a rapidly progressive disease which in untreated cases ends fatally within a few months or less (1 4). There have been few reports of immunosuppressive treatment of this primary renal disease—which lacks the systemic involvement found in Goodpasture's syndrome Wegener's granulomatosis polyarteritis nodosa and systemic lupus erythematosus—and no substantial evidence of improvement in renal function due to the immunosuppression has been reported (2 5 7). In the present investigation 10 patients with primary oliguric extracapillary glomerulonephritis were treated with prednisone and/or azathioprine which led to a marked improvement in renal function when the two immunosuppressives were administered simultaneously

MATERIAL

The material comprised 10 patients, 4 females and 6 males, aged 7–61 years. The clinical picture in all patients was very similar The initial complaints were arthralgias and gastrointestinal symptoms, and in no case were there such renal symptoms as haematuria, oedema or hypertension.

On admission the duration of symptoms ranged from 4 to 60 days. All patients were oliguric and uraemic Endogenous creatinine clearance was 0–3 ml/min and BP 125/80–190/120 mmHg. Clinical and laboratory data are given in Table I.

Antistreptolysin titre was increased (≥200) 3 patients (nos. 4 6 and 10) and normal (<200)

Pathological changes

Renal tissue was obtained from 9 patients by [illegible] and from one patient (no 1) at autopsy In [illegible] two biopsies were performed at an interval of 30 d. (Table II).

In the initial biopsies from the 10 cases crescents generally cellular were found in 60–100% of the corpuscles examined Collagen organization was present in [illegible] 50% of the crescents in cases 4 and 7 b only Recent changes comprising focal necrotic areas fibrin deposit or infiltration with polymorphonuclear leucocytes were frequently seen in both the crescents and the glomeruli In the latter there were locally slight basement membrane thickenings. Few or no hyalinized glomeruli were present. Some hypercellularity of the glomeruli was found in 9 of the 11 biopsies.

Slight to moderate tubular atrophy and slight interstitial fibrosis was seen in 3 biopsies In the interstitial tissue the presence of oedema and some infiltration with polymorphonuclear leucocytes and lymphocytes was more frequent finding. There were no signs of arteritis and no morphological changes consistent with collagen disease.

In patient 7 from whom the first biopsy was obtained just before the combined immunosuppressive treatment was initiated, crescent formation was found in 82% of corpuscles. None of the crescents were hyalinized. Necrotic changes were seen in both the crescents and the glomeruli. In the second biopsy the frequency of crescents was reduced to 48% of the corpuscles and in 4 of 11 crescents there were hyaline changes. At this time no necrotic lesions were observed in the crescents or the glomeruli (Fig. 1).

Treatment

Patients 1–3 were treated with prednisone 4 and 5 with azathioprine and 6–10 with prednisone and aza-

Table I *Immunosuppressive treatment*

Pat no	Sex	Age (y)	Duration of disease before treatment (d.)	Days before response or death	Endogenous creatinine clearance (ml/min) At start of treatment	1 mo. after treatment	At latest follow up	Proteinuria (g/24h) At start of treatment	1 mo. after treatment	At latest follow-up	Time of follow-up (mo.)
1	♂	19	30	12 (†)		–	–	0.1	–	–	
2	♂	45	45	15	2	27	52	1.5	3.2	1.1	43
3	♀	56	60	18 (†)		–	–	0.2	–	–	–
4	♂	9	45	45 (†)	2	–	–	1.8	–	–	–
5	♂	17	30	52 (†)		–	–	2.5	–	–	
6	♀	7	14	10		25	21	1.0	4.0	5.5	4
7	♂	32	30	14		70	75	0.4	0.7	2.1	15
8	♂	37	25	24	3	33	30	4.0	2.0	3.0	
9	♀	49	30	9		31	53	0.2	1.5	1.1	8
10	♀	61	4	15		30	48	0.3	2.0	1.8	13

thioprine (Table I). In the adult patients the initial dosage of prednisone was 80 mg/day and in the children 30 mg/day. Azathioprine was given in a dose of 2 mg/kg b.wt./day. At the most recent follow-up all responders were still on immunosuppressive treatment with azathioprine (2 mg/kg b.wt./day) and/or prednisone (5–30 mg/day).





Fig. 1. Renal biopsies from patient 7. In the initial biopsy (*a*) two corpuscles with cellular crescents are seen. Interstitial oedema is present between the tubules (H and E × 40). In the second biopsy one month later (*b*) slight interstitial fibrosis is found. In one of the two corpuscles the mesangial matrix is augmented and the crescent is hyalinized (H and E × 150).

Table II *Glomerular lesions*

+ = sparse, ++ = moderate, +++ = pronounced

	Crescents							Glomeruli				
Pat. no.	No of cor puscles	Frequency (%)	Epithelioid cell prolif eration	Fibrotic changes	Presence of poly-morphs	Fibrin deposits	Necrotic changes	Hyper cellu-larity	Presence of poly-morph	Fibrin deposits	Necrotic changes	Basement membrane thickening
1	>100	76	+++	+					+	+	+	+
2	35	86	+++	+			+	++	++	++	++	
3	10	60	++	+	+	+	+	+	+	++	++	+
4	96	100	+++	++			+	++				+
5	2	100	++	+				+	+	+	+	
6	20	95	++	+			+	+++			+	+
7	12	82	+++		+	+	++	+	+		+	+
7 b	23	48	++	++								+
8	26	69	+++				+	++	+		+	+
9	20	75	+++		++	++		+	++	++	+	
10	14	79	+++		+		+	++	+++		+	+

RESULTS

Only one (no ⌄) of three patients treated with *prednisone* responded to the treatment (Table I) At the most recent follow-up—43 months after *start of the treatment—this patient had an endo*genous creatinine clearance of 5? ml/mın a proteinuria of 1 1 g/?4 h and a normal BP

Both patients (nos 4 and 5) treated with *a a-thioprine* died without increased diuresis 45 nd 52 days after start of the treatment.

All 5 patients (nos. 6–10) treated with *prednisone and a.athioprine responded to the treat*ment in the course of 9–24 days. At the most recent examination—2–15 months after the favourable response—endogenous creatinine clearance was 21–75 ml/min, proteinuria 0.3–5.5 g/24 h and BP 120/60–220/120 mmHg

DISCUSSION

Except in a very few cases (3) ıt has gene been accepted that oliguric extracapillary gl ᵣ ulonephritis progresses to terminal uraemia in ł course of weeks or months (2, 4 6).

Only a few reports of immunosuppressı e treatment of oliguric extracapillary glomerulonephritı are available Thus Nakamoto et al (7) reported glucocorticoid treatment of olıguric glomerulonephritis At most 5 of their 9 patients suffered from the extracapillary type of glomerulonephritis and only 2 of these 5 patients responded to glucocorticoid treatment with improvement n reatı nine clearance Two patients with crescent formation" *in renal biopsy reported by Kincaid-Smith et* al (5) responded favourably to a combined treat ment with glucocorticoid azathioprine and anticoagulants. *However the percentage of corpuscles* showing crescent formation was not indicated and it is therefore difficult to make a comparison with our material.

In the present material crescent formation was found in 60% or more of the corpuscles Of the 3 patients treated with prednisone alone one responded with improvement in glomerular filtration rate (Table I) Both patients treated with azathioprine died f om uraemia. However all the 5 patients treated with a combination of prednisone and azathioprine achieved a substantial increase in creatinine clearance. There was no systematic difference in the severity of histological findings and clinical parameters including

evidence of antecedent streptococcal infection between the patients who responded to the treatment and those who died from apparently irreversible uraemia

In patient 7 from whom a second biopsy was obtained after one month of treatment, a reduction occurred in the percentage of corpuscles with crescent formation, as well as in other recent glomerular changes Thus in this patient the morphological changes paralleled the clinical course

In spite of the small number of patients investigated the uniformly favourable response in 5 consecutive patients treated with a combination of azathioprine and prednisone seems to indicate that the course of oliguric extracapillary glomerulonephritis can be improved by immunosuppression

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THE FUNCTIONAL PATTERN IN PATIENTS WITH RENAL DISEASE

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Abstract The glomerular filtration rate (GFR) the effective renal plasma flow (ERPF), and the filtration fraction (FF) have been studied in 38 patients with different renal diseases and varying degrees of renal insufficiency All diagnoses were verified by percutaneous renal biopsy Eighteen living kidney donors comprised a control material. In 10 patients in terminal uremia no characteristic functional pattern was found. Twelve patients with interstitial nephritis had a significantly increased FF 9 with the nephrotic syndrome had a significantly decreased FF whereas 7 with glomerulonephritis without the nephrotic syndrome did not demonstrate FF values different from those found in normals. A good correlation was found between the size of the FF and the concentration of serum albumin. Creatinine clearance overestimated the true GFR in patients with renal disease The FF was not significantly increased in hypertensive patients.

The development of clearance techniques in the thirties (22 23) which made it possible to evaluate glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) separately was followed by attempts to investigate whether various "renal disease entities" were accompanied by characteristic patterns of function. Most of these studies were carried out in patients with glomerulonephritis, pyelonephritis or nephrosclerosis and were undoubtedly motivated by an effort to contribute to diagnostic classification. In patients with post streptococcal glomerulonephritis a reduced filtration fraction (FF) was demonstrated (4 7) In patients with chronic pyelonephritis and minimal hypertension an increased FF was found (6) Similar observations were obtained in patients with hypertension, particularly malignant (9 10 13) Rasschou (17) found that an increased FF was not characteristic of patients with chronic pyelonephritis and hypertension. In general the results of these studies *did* not demonstrate any specific or characteristic functional patterns and therefore these physiological methods of study ha e never had any importance for clinical diagnosis

It should however be noted that diagnostic classification in these studies was made sole'y on the basis of clinical criteria and that these studies were carried out before the introduction of percutaneous renal biopsy Use of this technique [illegible] made an essential contribution to precise and clinical diagnosis in vivo

The purpose of the present study w [illegible] investigate the functional pattern in patients [illegible] different renal diseases and varying degree [illegible] renal insufficiency in whom diagnosis had bee[illegible] verified by percutaneous renal biopsy

MATERIAL

Fifty-six individuals were studied Eighteen of them were living kidney donors and served as control material (21) Of the 38 patients, aged 14–75 [illegible]ears, 21 were women and 17 men. All had renal disease and [illegible]iothalamate clearance varying from 1.[illegible] to 86 ml/min.

Patients were divided into the following groups Group I. 10 patients in terminal uremia of varying etiology (Table I) Group II. 12 patients with interstitial nephritis—of whom 9 had non-obstructive pyelonephritis without bacteriuria and 3 "analgetic" nephritis Group III. 9 patients with the nephrotic syndrome In 5 patients the basic disease was proliferative glomerulonephritis and in 2 membranous glomerulonephritis. In 2 patients minimal lesion or no change was found Group IV 7 patients with chronic glomerulonephritis without the nephrotic syndrome, of whom 4 had proliferative glomerulonephritis and 3 membranous glomerulonephritis. There were no patients with obstruction of the excretory renal tract.

Among the 28 patients in groups II–IV 14 had hypertension, of whom 7 in group II with interstitial nephritis, 3 were patients with the nephrotic syndrome and 4 with chronic glomerulonephritis. None of the [illegible]tients were clinically overhydrate[illegible] [illegible] ambulant. None received dialysis

Table III *Kidney function studies in patients with the nephrotic syndrome*
Abbreviations as in Table I

Pat. no.	Sex	Age (y)	C_{In}	C_{Cr}	C_{Cr}/C_{In}	C_{IH}	FF C_{In}/C_{IH}	FF C_{Cr}/C_{IH}	Proteinuria (g/24 h)	S-albumin (g/100 ml)	Hct (%)	BP
23	♀	49	11.0	16.3	1 48	63	17.5	.5 9	7.5	2.3	29	210/110
4	♂	28	18.9	27 9	1 48	87	1 7	32.1	10.0	2.2	36	170/130
25	♀	47	20.8	65 0	3.12	120	17.3	54.2	70 0	0.8	38	135/100
26	♀	32	28 3	48.4	1 71	178	15 9	27.2	17.5	2.4	35	190/105
27	♀	48	22 1	58.0	2 6.	277	8 0	20.9	19 0	0.5	35	140/100
28	♂	55	35 0	55.0	1.57	265	13..	20.8	15 0	1 0	31	120/80
29	♂	22	37 3	58.5	1.56	332	11.2	17 6	35 0	0.7	45	140/90
30	♂	54	43 0	73 0	1 70	440	9.8	16.6	11 0	0.5	29	120/70
31	♂	45	86.0	1.5 0	1 45	592	14.5	21 1	19.0	1 9	39	140/90
Average		42	33 6	58.6	1.85	261	14.3	26.3	17 0	1 4		
S D			22.1	30.6	0 60	174	4 3	11 6	8 0	0 8		
S E M			7 4	10.2	0 20	58 1	1 4	3.9	2.7	0.7		

chronic glomerulonephritis ($p<0.01$) Fourteen patients had hypertension 14 were normotensive FF among the hypertensives was 26.8%±9.5 whereas the value for the normotensives was 20 7%±9 6 The difference is not significant

DISCUSSION

Determination of GFR and ERPF in all groups showed good agreement between inulin and ¹²⁵I-iothalamate clearances and between PAH and ¹³¹I-hippuran clearances Thus renal handling of se materials appears to be identical even under hological conditions (8 19 20)

The reason for the change in relationship between GFR and ERPF could be damage to the glomerular apparatus changes in colloid osmotic pressure or lesions of the tubular apparatus (22). The high FF values in patients with interstitial nephritis show that damage to the tubuli dominates (Table II). PAH transport per nephron has been shown to increase with decreasing nephron population in non-uremic animals (3). If tubulus cells from rats and rabbits are treated with azotemic serum, the ability of these cells to accumulate PAH decreases (5 11 16) Since renal insufficiency as measured by ¹²⁵I-iothalamate clearance was identical in patients with interstitial nephritis and in patients with glomerulonephritis without the nephrotic syndrome one must accept that any effect of azotemic serum must be identical in the two groups As a whole it is questionable whether ¹³¹I-hippuran and PAH clearances are expressions of "effective" renal plasma flow in pa-

Table IV *Kidney function studies in patients with chronic glomerulonephritis*
Abbreviations a in Table I

Pat. no.	Sex	Age (y)	C	C	C_{Cr}/C_{In}	C_{IH}	FF C_{In}/C_{IH}	FF C_{Cr}/C_{IH}	Proteinuria (g/24 h)	S-albumin (g/100 ml)	Hct (%)	BP
32	♂	38	8.8	18 0	2 05	38	23 1	47 4	4.4	3.5	31	175/125
33	♀	56	9 5	16 7	1 76	41	23.2	40.7	1.5	3.6	24	120/90
34	♀	34	10 4	18 2	1 75	36	28.9	50 6	2.9	–	28	190/125
35	♂	50	13.5	23 8	1 76	98	13.8	4.3	2.5	3.4	44	170/125
36	♂	23	14 0	25.5	1 83	46	30.4	55 4	1..5	2.3	30	160/110
37	♀	22	16.4	32.0	1 95	96	17 1	33.3	4 0	..8	30	160/100
38	♀	26	37 6	50.0	1 33	157	24.0	31 9	0.0	3.2	47	115/85
Average		36	15 7	26.3	1 78	73 1	22 9	40.5	4.0	3 1		
S.D.			10.0	11 7	0..3	45 7	5 9	11 3	4 0	0.5		
S.E.M			3.8	4 4	0 09	17 3	2..	4.3	1.5	0.3		

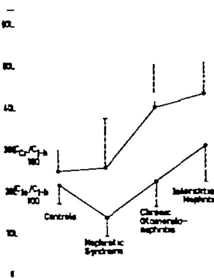


Fig 3 Relationship between creatinine, ¹²⁵I-iothalamate and ¹³¹I-hippuran clearances in controls and patients with kidney diseases.

tients with severe renal insufficiency as self depression takes place at a lower serum concentration. This self-depression depends partly on the degree of tubular destruction (14 18).

The low FF values in patients with the nephrotic syndrome could be a result of damage to the glomerular membrane and a low colloid osmotic pressure. Evidence that the low colloid osmotic pressure affects the FF can be seen in Fig. .., which shows a correlation between concentration of serum albumin and the FF In two patients with the nephrotic syndrome without glomerulonephritis and excessively low serum albumin, the same low FF was found The FF level was independent of whether the glomerular lesion was of proliferative or membranous type A normal or reduced FF value was found in all patients with glomerular lesions. The present investigation did not allow evaluation of which of the three factors —glomerular lesion tubular lesion, or changes in the colloid osmotic pressure—had the greatest influence on the functional pattern.

In this study BP had only a slight influence on the FF level. Half of the patients in groups II, III and IV were hypertensive The mean FF was somewhat higher in the hypertensives but a significant difference was not found

In the controls there was no significant difference between creatinine and ¹²⁵I-iothalamate clearances In groups II, III and IV creatinine clearance was significantly higher than ¹²⁵I-iothalamate clearance in all cases. In patients with the nephrotic syndrome the creatinine clearance values were on an average 85% higher than the ¹²⁵I-iothalamate clearance values which means that 42% of the amount of creatinine excreted was eliminated by a mechanism other than glomerular filtration.

Thus under pathological conditions creatinine does not meet requirements for a substance which alone is excreted by glomerular filtration (2, 1. 20) In patients in terminal uremia there is no longer any relationship between the nature of the renal disease and the level of the FF

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LACTOFERRIN AND IRON ABSORPTION IN THE SMALL INTESTINE

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Abstract. Lactoferrin (LF), an iron-binding protein, has been studied in (1) duodenal fluid of 16 hematologically normal patients, (2) duodenal fluid of 9 patients with iron overload (idiopathic and secondary hemosiderosis), (3) T-drain bile of 14 cholecystectomized patients. Patients with iron overload show on an average lower LF values in the duodenal fluid than normal individuals. This relation with iron metabolism is also present in the group of normal controls, higher iron and iron saturation levels of the plasma are accompanied by lower LF values. In the first days after cholecystectomy this relation is lost. Purified human LF brought into the duodenum, distinctly inhibits iron absorption in the intestine during the first hours of absorption. However this effect is absent in patients with idiopathic hemosiderosis.

Lactoferrin (LF) is an iron-binding protein, originally discovered in cow-milk by Sørensen and Sørensen (28) but later also found in other external secretions such as bile pancreatic fluid and small intestinal secretions of many species including man (3 4 10 12 17 18 19 20 26 27)

LF has much resemblance to transferrin (TF) Both proteins are composed of a single polypeptide chain (1 7), have a nearly identical molecular weight of approximately 80 000 and can bind metal atoms to two specific metal-binding sites of each molecule

Human LF is saturated with iron at a metal. protein ratio of about 0 025 μmoles/mg protein (20) but in its native form in milk LF was found to be saturated with iron to about 20% of its iron-binding capacity (17) The LF–iron complex is very stable between pH 4 and 11 Between pH 4 and 2 the complex is even more stable than the TF–iron complex (17)

Human LF has a pronounced tendency to form complexes with more acid proteins for instance casein or albumin (11) In vitro it could be proved (11) that these interactions influence the electrophoretic behaviour of LF Electrophoresis of the isolated protein in agarose reveals migration to the γ-region in contrast to the naturally occurring LFs which show a line of precipitation in the β-region

Though there are many similarities between LF and TF the two proteins show distinct differences when one studies their antigenic properties, amino acid compositions, carbohydrate moieties and tryptic peptide patterns (4 20 21) Furthermore LF is normally not detectable or only in very low concentrations in blood plasma by radioimmunoassay (25)

Fo LF as well as for TF two possible functions can be considered. (1) Bacteriostatic function LF not saturated with iron shows in vitro a bacteriostatic effect that is abolished if LF is saturated with iron (6 17 18 19 20 27). (2) One can consider the possibility of role of LF in iron transport in the intestine because LF is found in considerable quantities in gallbladder bile and small intestinal fluid

There are only a very few studies on the influence of LF in bile pancreatic fluid and small intestinal secretions on iron absorption (5 13) We therefore studied in more detail LF concentrations in bile of patients with a choledochus drain after cholecystectomy and also compared LF concentrations i duodenal fluid of hematologically normal patients and patients with idiopathic or secondary hemosiderosis. We investigated the relations between LF and some parameters of iron metabolism Furthermore we studied the influence of intraduodenally applied purified LF on the first hours of iron absorption

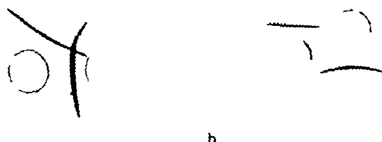


Fig 1 Immunodiffusion in agar (*a*) Upper well = human milk, left bottom well = rabbit antiserum against human LF right bottom well = rabbit antiserum against human TF In human milk LF as well as TF are present. The precipitation lines show a picture of non-identity

(*b*) Upper well = human ferritin, bottom well = human LF left well = rabbit antiserum against human ferritin, right well = rabbit antiserum against human LF N cross-reactions are observed

MATERIAL AND METHODS

Isolation of lactoferrin from human milk

LF was prepared from human milk by centrifugation at 5000 *g for 20 min to separate the fat from the milk* and afterwards at 20000 *g* for 1 hour at 4°C in order to remov casein.

The supernatant is saturated with iron by addition of 1 ml FeSO 7H$_2$O solution (containing 1 mg Fe^{++}/ml) to every 100 ml. After standing for 4 hours a red colour develops. Most of the proteins of the supernatant precipitated by stepwise salting out with ammonium ate at pH 8 0 and removed by centrifugation at *g* for 5 hou LF remains in the supernatant till monium sulphate concentration has reached 45% en the remaining proteins including LF precipitate

After redissolving, the precipitated crude product is dialysed against running tapwater for 24 hours and afterwards applied to a column of DEAE-cellulose equilibrated with a phosphate buffer pH 7.4 LF was eluted by the same buffer using stepwise increased concentrations from 0.005 M to 0.05 M. After pooling, the fractions containing LF were applied to a Sephadex G-200 column, equilibrated with Tris-HCl 0.1 M in 1 M NaCl, pH 8.0. LF free of detectable contaminants, was eluted by means of the same Tris-HCl buffer

On an average 100 mg pure LF was prepared from 100 ml milk. This isolated salmon-pink protein contain 0.11% iron. It can bind radioactive iron as could be proved by autoradiography

Immunoelectrophoresis using rabbit antiserum against human serum proteins (Nordic Pharmaceutical and Diagnostics Tilburg, The Netherlands) did not reveal any serum contaminants in our LF samples

Preparation of antisera

Antiserum against LF was prepared in rabbits by four i.m. injections of 0.5 mg LF emulsified in 0.5 ml phosphate buffer pH 7 4 administered at intervals of 14 days. On the first two occasions complete Freund's adjuvant was added to the emulsion. The antiserum mostly revealed only one precipitation line with LF but sometimes with human milk one or two faint extra precipitation lines appeared

After absorption with pooled human serum these contaminants were no longer detectable Especially we obtained no cross reactions with TF and ferritin (Fig. 1 and *b*).

Antiserum against human TF (Nordic Pharmaceuticals and Diagnostics Tilburg, The Netherlands) was prepared in a rabbit in the same way as antiserum against LF After absorption of this antiserum with albumin and acid seromucoid fraction only one precipitation line with human TF and human serum was obtained

Quantitative determination of lactoferrin transferrin and IgG

In bile and duodenal fluid LF TF and IgG were determined by the single diffusion test (15 16) For quantitative determination of IgG we used commercially available agar-gel plates with specific IgG antiserum (Partigen Immunodiffusion plates, Behringwerke Marburg/Lahn W Germany)

Iron determination in serum bile and duodenal fluid

The serum iron concentration and total iron-binding capacity (TIBC) were measured by colorimetric determination with bathophenanthroline disulphonate in deproteinized plasma (24 29) The iron concentration in bile and duodenal fluid was measured after wet ashing (14) by colorimetric determination as above (24, 29). In both determinations we used the commercially available iron test combination of Boehringer Mannheim, W Germany

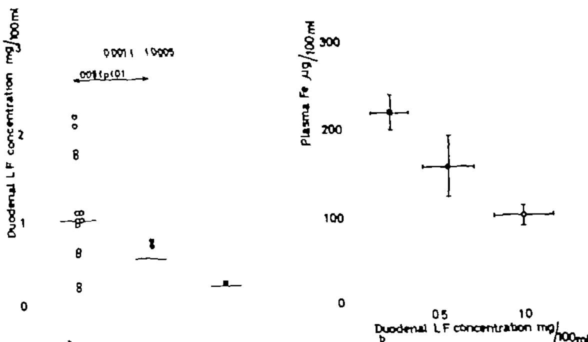


Fig. 2. (a) LF concentration in non-stimulated duodenal fluid. ○ = 16 controls (mean LF concentration 1.0 mg/100 ml), ● = 5 patients with idiopathic hemosiderosis (mean LF concentration 0.56 mg/100 ml), ■ = 4 patients with transfusion hemosiderosis (mean LF concentration 0.22 mg/100 ml). (b) Plasma iron and duodenal LF concentration of the three groups of patients (mean ± S.E.M.).

Determination of radioactivity

Radioactive iron ($^{59}Fe^{2+}$-ascorbate and $^{59}Fe^{3+}$-citrate) was obtained from the Radiochemical Centre, Amersham, UK. The radioactivity of ^{59}Fe in plasma was counted with sodium iodide well-type crystal. The ^{59}Fe plasma samples were mixed with suitable amounts of insta-gel (Packard) and counted in liquid scintillation counter.

Determination of iron absorption in the whole-body counter

Absorption and retention of ^{59}Fe in two patients with idiopathic hemosiderosis were also measured in the whole-body counter of the Radiological Department, Health Organization, Bilthoven, The Netherlands.

Selection of patients

The following groups of patients were studied:

I. In 14 patients we continuously sampled bile delivered by a T-drain after cholecystectomy. All patients had normal liver functions. None was jaundiced. No blood transfusions were required during or after operation. The following measurements were made: *Blood* Hb concentration (g/100 ml), Hct (%), serum iron concentration (μg/100 ml), TIBC and latent iron-binding capacity (LIBC) (μg/100 ml), iron saturation (%). *Bile* LF (mg/100 ml and mg/24 hours), TF (mg/100 ml and mg/24 hours), IgG (mg/100 ml and mg/24 hours).

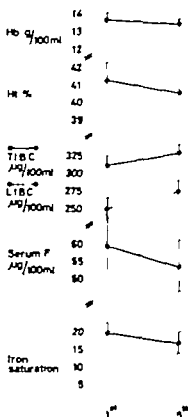
II. Sixteen hematologically normal patients were selected for measurement of LF concentrations in non-stimulated duodenal fluid obtained by duodenal intubation. These patients had normal liver function tests and no pancreatic or gallbladder disease.

III. The same study was performed in 9 patients with iron overload. In 5 of them idiopathic hemosiderosis was present as proved by liver biopsy, desferrioxamine test, family history and other clinical parameters. In 4 patients a secondary hemosiderosis due to blood transfusions during chronic hemodialysis existed.

IV. In 11 hematologically normal controls (not the same individuals as studied in group II) and in 5 patients with an idiopathic hemosiderosis (already mentioned in group III) we studied the effect of purified human LF on the initial plasma radioactivity after duodenal administration of two tracer doses of radioactive iron, one with and one without 25 mg LF.

As human gallbladder contains on an average 10–30 mg LF a quantity of 25 mg, as used in our experiments can be considered a physiological one.

The composition of the test solution was: 8 μCi $^{59}Fe^{3+}$-citrate or $^{59}Fe^{2+}$-ascorbate with 10 μmoles Fe^{2+} as $FeSO_4 \cdot 7H_2O$ as carrier and 5 ml 0.1 M citrate buffer pH 6.2. This solution was brought directly through tube into the duodenum in order to prevent denaturation of LF by gastric juice. We administered LF alternately with the first and the second tracer active iron in order to avoid systematic of instrumentation. The twofold



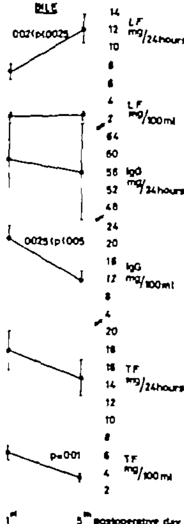


Fig 3 Comparison of data from the 1st and 5th post operative days in 14 cholecystectomized patients (mean values)

active tracer doses to the same patient once with and once without LF eliminates individual differences in iron absorption

Statistical methods

Differences in iron concentration radioactivity and LF concentration were tested for statistical significance by the Student t-test (8)

RESULTS

1 Lactoferrin in Non-stimulated Duodenal Fluid

Studying the relation between iron metabolism and LF we compared the LF concentration in non-stimulated duodenal fluid of 16 hematologically normal control patients 5 patients with an idiopathic hemosiderosis and 4 with a secondary hemosiderosis All patients with idiopathic and secondary hemosiderosis had a LF concentration in the duodenal fluid lower than 1 mg/100 ml

In the group of hematologically normal control patients a great variability of the LF concentration in the duodenal fluid exists but the difference in mean value between the normal and iron-loaded patients is highly significant ($p<0.01$) Our findings are shown in Fig a

Considering the difference in LF concentration in normal individuals and patients with iron o er load we studied in the group of normal controls the relation between LF and some parameters of iron metabolism The high variability of LF in normals proved also to be related to the iron status high plasma iron and iron saturation proved to be associated with low LF values (plasma iron – duodenal LF $r=-0.57$ $p=0.01$ $n=16$ iron saturation – duodenal LF $r=-0.63$ $p=0.005$ $n=$

16). A higher TIBC is accompanied by a higher LF concentration ($r = +0.51$ $0.01 < p < 0.025$ $n = 16$).

Thus these findings point in the same direction as our findings in patients with iron overload though in the latter a more extreme situation exists This is illustrated in Fig. 2 *b*

II *Lactoferrin Concentration in Bile after Cholecystectomy*

To study the relation between iron metabolism and bile LF we also investigated 14 patients during the first 5 postoperative days after cholecystectomy

The mean values of the results on the first and fifth postoperative days were compared and are illustrated in Fig. 3 We chose these days because a fairly steady state exists from the fourth postoperative day and oral iron uptake is negligible up to the sixth day Also excretion of IgG and TF in the bile are constant from the fourth postoperative day (Fig 4)

As expected the mean values of Hb concentration Hct and serum iron show a slight decrease postoperatively while TIBC and LIBC increase These changes however are not significant. At the same time the 24-hour LF excretion in bile increases significantly whereas the concentrations and the 24-hour excretions of TF and IgG in bile decline A typical example of our findings is presented in Fig. 4

From Figs 3 and 4 it may be concluded that in the first days some plasma leakage occurs in the bile ducts very probably caused by surgical manipulation and slight inflammatory reaction resulting in biliary TF and IgG loss and diminishing on the next postoperative days LF however shows a rising biliary excretion during these days thus demonstrating an opposite effect to TF Apparently the two proteins in bile behave differently (30)

Also i this group of patients we analysed the relation between biliary LF and some parameters of iron metabolism The correlations (r) are presented in Table I From Table I we can conclude that the inverted relation between iron content and LF found in the duodenal fluid of normals and patients with iron overload is lost in cholecystectomized patients

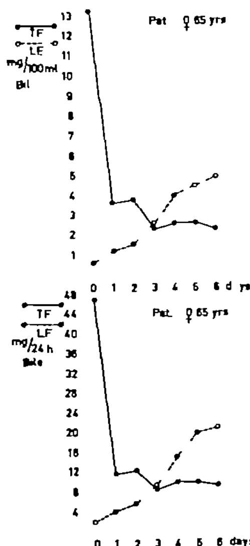


Fig 4 LF and TF in bile of an individual patient after cholecystectomy *Top.* LF and TF concentration Bottom: 24-hour LF and TF excretion of the same patient.

III *Effect of Lactoferrin on Iron Absorption*

(a) Hematologically normal control patients

The laboratory data of 11 hematologically normal control patients (group IV) are summarized in Table II

In all patients of this group except one the plasma radioactivity 1 2 and 3 hours after the test dose of radioactive iron is lowered by administration of 25 mg LF into the duodenum (Fig 5)

Plasma radioactivity i expressed as per

Table I Correlation between LF in bile and plasma iron iron saturation Hb concentration and Hct on the first and fifth postoperative days

	LF in bile			
	1st day		5th day	
Blood	mg/100 ml	mg/24 h	mg/100 ml	mg/24 h
Hb (g/100 ml)	+0.72	+0.68	+0.60	+0.63
Hct (%)	+0.45	+0.48	+0.51	+0.63
Fe (μg/100 ml)	+0.72	+0.60	n.s.	n.s
Fe saturation (%)	+0.62	+0.57	n.s.	n.s.

Only significant correlations ($p < 0.05$) are presented n.s.=non-significant.

centage of the oral dose of radioactive iron The inhibitory effect of LF on the plasma radioactivity can be calculated by the ratio of the plasma radioactivity after an oral dose of radioactive iron with and without LF This ratio is expressed as [Fe(+LF)/Fe(−LF)]×100% The average plasma radioactivity measured 1 2, and 3 hours after the test dose of 25 mg LF is considerably diminished to respectively 79.5% 41 4% and 53 4% of plasma radioactivity after the same test dose without LF (Table III Fig 6)

One patient (case 9) suffering from a chronic gastritis, reacted completely differently from the other patients for reasons yet unknown. Careful clinical investigation revealed no other abnormalities liver biopsy was refused so the iron content of the liver remained unknown Control experiments performed with 25 mg albumin ($n=3$) 25 mg TF ($n=3$) and 25 mg IgG ($n=3$) showed no inhibitory effect on the plasma radioactivity curve

(b) Patients with idiopathic hemosiderosis

The same investigation was made in 5 patients with idiopathic hemosiderosis The hematological data from these 5 patients are summarized in Table II All patients showed a positive desferrioxamine test and iron accumulation in liver biopsy As expected there is a large variability in the hematological data of this group because some patients had been treated by phlebotomy very recently

There is no distinct inhibitory effect of LF on

II *Hematological data from normal controls and patients with idiopathic hemosiderosis*

	Sex	Age (y)	Fe (μg/100 ml)	TIBC (μg/100 ml)	Saturation	Hb (g/100 ml)	Hct (%)	Last phlebotomy
Normal controls								
1	♂	48	114	299	36	14.8	45	
	♂	22	94	297	29	14.2	44	
3	♂	38	85	284	30	14.3	44	
4	♂	42	76	270	26	14 4	42	
5	♂	48	137	285	47	17.8	53	
6	♀	74	109	313	34	13 8	42	
7	♂	30	115	370	32	14 4	44	
8	♂	30	110	320	34	14 7	45	
9	♀	41	88	366	23	14.2	41	
10	♂	24	184	297	64	16 8	51	
11	♂	45	150	312	48	14 9	46	
Patients with idiopathic hemosiderosis								
1	♂	48	200	280	71	16.8	50	1 y
2	♂	46	43	276	16	14.5	44	1 y
3	♂	62	120	282	47	16.0	50	5 d
4	♀	45	225	40	97	14.8	46	14 d
5	♂	57	10	390	60	15.5	48	1 mo

All patients with idiopathic hemosiderosis showed positive liver biopsy with iron accumulation and a positive desferrioxamine test.

the plasma radioactivity curve (Table III Fig 7) The difference between the controls and the patients with idiopathic hemosiderosis with respect to the effect of LF on the plasma radioactivity in the first hours after a test dose of radioactive iron is highly significant After 3 hours there is a non-significant difference (Fig. 6)

Two of these patients were investigated with whole-body counting 10 and 14 days after the test dose of ^{59}Fe with and without LF Both patients had as expected a very high iron retention after 10 and 14 days Here we found no significant difference between the iron retention after a test dose with and without LF (Fig 7).

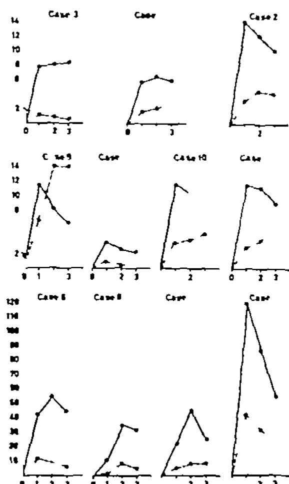


Fig 5 Plasma radioactivity curves of 11 normal control patients. Plasma radioactivity after an oral tracer dose of 25 mg LF is always lower than after the same tracer dose without lactoferrin, except in one patient (no 9) =radioactivity/100 ml plasma (percentage of the oral tracer dose 10^{-4}), =time (h), ●—●=^{59}Fe without LF ●- -●=^{59}Fe with LF

DISCUSSION

Very few data are available in the literature about the relation between the iron-binding protein lactoferrin and iron absorption in the small intestine

Blanc et al. (5) determined the influence of LF on intestinal iron absorption in two patients, in one of whom they found a significant decrease in absorption. However a slight increase was shown in the red cell uptake of radioactive iron in both patients after 10 days. De Laey et al (13) studied the effect of human LF on iron absorption in rats using everted duodenal sacs (16). They concluded that exogenous apo-lactoferrin (iron-deprived protein) significantly decreased the mucosal iron uptake but iron-saturated LF did not much influence iron uptake in the mucosal cell The accumulation of iron in the duodenal mucosa of guinea pigs was significantly higher after pre-incubation of duodenal loops with LF antibodies From these experiments they concluded that LF is of importance for the control of iron absorption in the small intestine

Our results also suggest a relation between LF in bile and duodenal fluid and iron metabolism in man. In normal individuals higher values for plasma iron and plasma-iron saturation are associated with lower duodenal LF concentration and vice versa. In patients with iron overload in idiopathic hemosiderosis as well as in secondary hemosiderosis, the duodenal LF is low representing a more extreme example of the relations already found in hematologically normal individuals

Now the question arises whethe higher iron saturation of the body especially the liver causes the low LF excretion in the bile or whether the causal relation is the reverse in the sense that LF poured out into the duodenum, inhibits iron absorption Our studies with purified LF brought into the duodenum are very suggestive of the latter view The inhibition of iron absorption is very significant in the first hours of iron absorption. The LF dose we used is a physiological one The gall bladder contains ±10–30 mg LF and each contraction of the bladder pours out such a quantity of LF into the duodenal lumen.

We realize that the plasma radioactivity after iron isotope administration is only an indirect

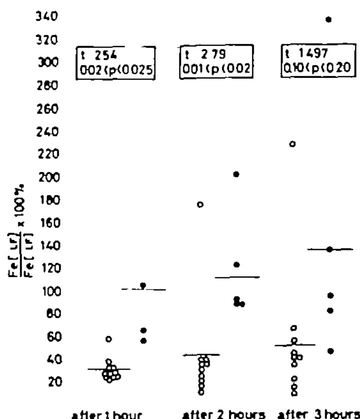


Fig 6 Comparison of the inhibitory effect of LF on the plasma radioactivity after an oral tracer dose of radioactive iron in normal controls (O) and patients with idiopathic hemosiderosis (●). After 1 and 2 hours a statistically significant difference between the two groups is observed. After 3 hours there is no significant difference

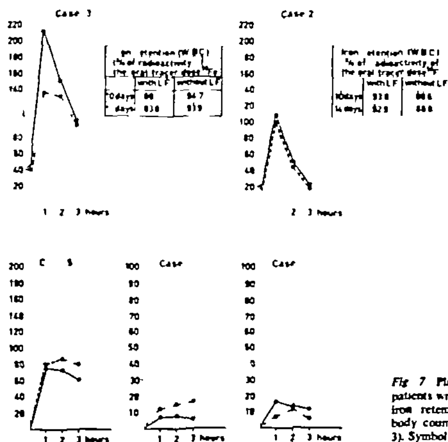


Fig 7 Plasma radioactivity curves of 5 patients with idiopathic hemosiderosis and iron retention determination by whole-body counting in 2 patients (nos. 2 and 3). Symbols as in Fig. 5

Table III *Inhibitory effect of lactoferrin on plasma radioactivity in normal controls and patients with idiopathic hemosiderosis*

Pat. no.	1 hour (%)	2 hours (%)	3 hours (%)
Normal controls			
1	36	35	4_
	21	37	40
3	26	10	6
4	26	15	40
5	30	30	53
6	76	–	1
7	23	18	33
8	4	23	16
9	57	173	226
10	30	38	–
11	25	35	66
Mean	29.5	41.4	53.4
Patients with idiopathic hemosiderosis			
1	40	85	44
2	91	88	80
3	64	86	93
4	201	200	333
5	104	120	133
Mean	100.0	115.8	136.6

measure of the initial iron absorption and that the inhibition we found does not mean that ultimate iron retention is also diminished Further studies will be necessary to elucidate the influence of LF on iron absorption in the lower parts of the intestine LF cannot in our view exert its inhibitory effect on initial iron absorption by molecular binding of iron ions because we used nearly iron-saturated LF (80%).

As yet one can only speculate about the mechanism of this inhibitory action The special affinity of LF for other proteins may be of importance to this phenomenon. LF is a peculiarly sticky protein and one can consider the possibility that it blocks mucosal iron receptors of a protein nature

A very interesting point in connection with this hypothesis is our finding that in primary hemosiderosis this blocking effect of LF is absent These patients have a very low LF concentration in their duodenal fluid but its blocking effect is lost too Whether this is an essential feature of primary hemochromatosis we cannot say It could be a result of iron accumulation in the mucosa. Studies in secondary hemochromatosis could help to solve this problem, but unfortunately the absorption in these cases is so low that further inhibition by LF cannot be clearly demonstrated But perhaps biliary LF is a protective system against too high iron absorption which is defective in primary hemosiderosis

Our studies of biliary LF in cholecystectomized patients also require some discussion. Here the normal relations between some parameters of iron metabolism and lactoferrin are lost. Shortly after operation plasma iron iron saturation Hb and Hct are positively correlated with biliary LF concentration instead of the negative correlation we found in normal individuals

On the other hand the mean value of the 4-hour biliary LF excretion in the cholecystectomized group shows a significant rise during the next postoperative days together with a non significant fall of Hb iron concentration and iron saturation of the plasma suggesting again the same relation as we found in normal individuals.

An explanation of all postoperative findings is hampered by the fact that surgical intervention and stress strongly influence plasma iron and TF which very probably are trapped in the RES (22, 23) Other complicating factors are blood loss release of iron from hematoma combined with very reduced oral iron uptake loss of iron and LF through the T-drain and postoperative variations in bile flow Another disturbing factor may be slight liver dysfunction due to anesthesia. Furthermore biliary LF values before operation are unknown

We think, therefore that cholecystectomized patients are not a suitable model for studying exactly the relations between biliary lactoferrin and iron metabolism.

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ITCHING IN PREGNANCY

A 15-year Follow-up Study

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Abstract All pregnancies in 89 women wo had had itching during pregnancy have been characterized on the basis of personal interview and available hospital records. Most of the non-cholecystectomized women underwent an oral cholecystography The majority of the women were between 35 and 55 years old The study was performed approximately 15 years after their last complicated pregnancy Itching had recurred during subsequent pregnancies in 34 of the 71 women with more than one full-term pregnancy Delivery tended to occur earlier in pregnancies with itching (median 40th week) than in uncomplicated ones (median 41st week). Children born at deliveries with itching were somewhat lighter than those born after uncomplicated pregnancies (median 3365 and 3500 g). Meconium staining of the amniotic fluid was observed in 27% of deliveries with itching and in 9% of those without. Four children born to women with recurrent itching died from asphyxia at or shortly after birth. Two of the pregnancies had been complicated by itching. Evidence of earlier (cholecystectomy) or present (abnormal cholecystography) cholelithiasis was found in 35 women The minimal prevalence was higher in omen with recurrent itching (59%) than i the remainder (27%) The combined evidence from this and previous study indicates that the minimal prevalence of cholelithiasis in women 35–60 years old with history of recurrent cholestasis of pregnancy is 58% The figure is significantly higher ($p<0.001$) than that encountered in women of comparable age in two Swedish autopsy series.

Cholestasis of pregnancy is a syndrome characterized by itching with or without jaundice It usually appears in the last trimester and the symptoms disappear at or shortly after delivery (12) Several reports demonstrate that cholestasis of pregnancy is associated with a slightly shortened gestation (4 5 8 12, 13 19 20 22 30 31) a subnormal birth weight of the child (9 24 25 30) and a high incidence of fetal ... 4 25). The cause of cholestasis of pregnancy is not known. A recent study demonstrated a familial predisposition in women with recurrent symptoms during several pregnancies (10) These women furthermore developed gallbladder disease to an unexpectedly high extent (11)

The reports cited above concern women with cholestatic jaundice of pregnancy The milder and probably most common form of cholestasis of pregnancy (26) is characterized by itching in the absence of jaundice (1) The object of the present study was to investigate whether the observations referred to above are valid for women with pruritus during pregnancy as the dominant symptom.

MATERIAL AND METHODS

From March 1955 to Feb 1956 Arfwedson, who had been the first to prove the association between generalized itching of pregnancy and liver dysfunction (2), investigated 100 women with itching during pregnancy randomly selected among the patients admitted to the Department of Obstetrics of Malmö General Hospital in the south of Sweden. With the permission of Dr Arfwedson and with access to the names birth dates and hospital record numbers of the women, 98 of them could be traced through population registers

The present study included an interview by telephone, following standard questionnaire. Further information was obtained from hospital records. Most deliveries (216 of 49) took place at Malmö General Hospital. During Arfwedson's investigation notes were made in the delivery records of the presence or absence of itching and jaundice during the current and previous pregnancies. In delivery records written *after* Arfwedson investigation the presence of itching was usually noted as complication of pregnancy The recollections of the women regarding the presence or absence of itching and jaundice usually

Table I. Number of pregnancies in relation to recurrence of itching

		Full-term pregnancies		Abortions		Total	
Group	No. of women	With itching	Without itching	With itching	Without itching	With itching	Without itching
1	34	108	13	0	18	108	31
	37	36	74	1	16	37	90
3	18	18		0	8	18	8
Total	89	16_	87	1	42	163	1?9

the delivery records but in some cases there was differenc concerning the duration of these symptoms. When record were missing, the interviews were sometimes the only source.

All information regarding complications during pregnancy other than itching and jaundice the date of the last normal menstruation maternal and fetal complications during deli ery and the birth weights of the children, were obtained from delivery records. Other data related below originate from the interviews. The lengths of pregnancies were calculated from the last normal menstruation. The severity of itching and jaundice was roughly graded as described previously (8).

Non-cholecystectomized women were referred to their local hospital for a cholecystography unless such an examination had been performed within the last 5 years.

The χ^2-test was used in the statistical calculation.

RESULTS

Eighty-nine of the initial 100 women took part in he study. Two women had died (one from carcinoma of the breast the other in an accident) others could not be traced and 7 were unwilling to participate.

The women were divided into 3 groups according to the recurrence of itching during pregnancy.

Table II. *Age ( ) of the women at the time of the first pregnanc (I) at th last pregnancy ith itching (II) and at th follow up study (III) (mean and range)*

Group	No. of women	I	II	III
1	34	23 17–35	3? 1 22–42	44 3 34–59
2	37	23.5 18–31	28 1 18–40	45 1 35–58
3	18	27 4 20–37		44 4 37 54

Group 1 comprised 34 women who had had this symptom during more than one pregnancy. Group 2 included 37 women with itching during one of several full-term pregnancies. The 18 women in group 3 had had only one full-term pregnancy. There were 29? pregnancies in all 43 of which ended in abortion (Table I). One abortive pregnancy (group 2) was complicated by itching.

The mean age of the women at their last pregnancy complicated by itching was about 30 years. The follow-up study was performed approximately 15 years later (Table II).

The pregnancies

Group 1. Of the 1 1 full-term pregnancies of the 34 women 13 seem to have been free from pruritus (Table I). Itching always ended at or within a few weeks after delivery. The median duration was about 2 months (range 0.5–7.5). In 20 pregnancies of 11 women there had been visible jaundice in association with itching. The duration of symptoms was somewhat longer in these pregnancies than in those with itching alone (2.5 and 1.5 months respectively). Jaundice was observed about 1 month (median value) after itching had started and ended at delivery. Pregnancies not complicated by itching occurred before after or between pregnancies with itching, in no discernible pattern.

Group 2. The 37 women underwent 110 full-term pregnancies. The median duration of itching was 1 month (range 1 week – 7 months). Itching usually ended at or shortly after delivery but in 6 pregnancies it disappeared 0.5–3.5 months (median _.5) before delivery. Itching occurred together with jaundice in 2 pregnancies.

Group 3. None of the 18 women had jaundice during pregnancy. The median duration of itching was 2 months (range 1 week – 4 months). Itching disappeared before delivery in pregnancies

Table III. *Length of pregnancies (median and range)*
No. of deliveries ending before the 37th week within parentheses

		Itching at delivery		Itching ending before delivery		No itching	
Group	No. of women	No.	Week of delivery	No	Week of delivery	No.	Week of delivery
1	34	88 (10)	40 31–46			9 (3)	39 26–42
2	37	23 (2)	41 34–43	5 (1)	40 36–41	65 (2)	41 35–45
3	18	16 (4)	40 31–44	2	38 39		
Total	89	127 (16)	40	7 (1)	40	74 (5)	41

(2.5 and 4.5 months) In all other instances it vanished at or shortly after delivery

Hypertension (diastolic BP≥95 mmHg) and/or proteinuria had been found in 25 women during 33 pregnancies The incidence of proteinuria and/or hypertension was approximately the same in all groups, and was not associated with the presence of pruritus

There is no indication that any of the women had been exposed to infectious hepatitis prior to a period of itching Urinary tract infection was observed in 11 women during 12 pregnancies 9 of which were also complicated by itching. Five women were referred to group 1 5 to group 2 and 1 to group 3 Four women (group 2) were operated on for appendicitis while pregnant. Two of them developed itching, in one case with jaundice a few days after the operation. The other 2 had no itching during these pregnancies.

The deliveries

Records could be traced from 243 of the 249 deliveries. There were 4 twin deliveries (1 in group 1 3 in group 2) which are excluded from the following account. All twin pregnancies were complicated by itching up to delivery

The date of the last normal menstruation was given in 208 records covering deliveries with a spontaneous onset. Delivery occurred in the 40th week (median value) in the 134 pregnancies complicated by itching, and in the 41st week in the 74 without itching (Table III) There was no obvious difference in this respect between pregnancies with itching ending before delivery as opposed to after In the 11 women in group 1 who had had jaundice delivery occurred in the 39th week (median value) in complicated pregnancies

In 22 women in group 2 the complicated pregnancy tended to be shorter than the mean of the woman's uncomplicated pregnancies (mean difference 1 week) In individual women with more than one complicated pregnancy (group 1) there was no correlation between the severity of itching (or the presence of jaundice) and the length of pregnancy

An estimation of the blood loss during delivery was given in 234 records. When uterus-contracting drugs (secale alkaloids or oxytocin preparations) were used, the median blood loss in deliveries with itching was 150 ml and 190 ml in uncomplicated deliveries. In the remaining deliveries the median blood loss was 200 ml whether there had been itching or not. There were no discernible differences between women referred to different groups nor between complicated and uncomplicated deliveries in individual women.

The children

The birth weight was registered for 223 children (twins excluded) born at spontaneous deliveries Children born at deliveries complicated by itching were somewhat lighter than children born after uncomplicated pregnancies (median weight 3365 and 3[illegible]00 g respectively) (Table IV)

In individual women with recurrent itching (group 1) there was no correlation between the severity of symptoms (or the presence of jaundice) and the birth weight of the child In the 24 women in group 2 (i.e. no recurrent cases) with

Table IV *Birth weights (g) of the children in relation to itching during pregnancy (median and range)*
No. of premature children (birth weight ≤2 500 g) within parentheses

		Itching at delivery		Itching ending before delivery		No itching	
Group	No. of women	No.	Birth weight	No	Birth weight	No.	Birth weight
1	34	93 (7)	3 350 340–4 560	–		10 (2)	3 700 1 020–3 900
	37	26 (1)	3 410 030–4 250	5	3 260 2 900–3 680	7? (?)	3 530 2 200–5 330
3	18	15 (2)	3 380 1 860–4 350		3 550 3 860	–	
Total	89	134 (10)	3 365	7	3 400	82 (4)	3 500

ing during delivery the child born at that delivery tended to have a slightly lower birth weight than the mean of its siblings (mean difference ?00 g)

Four children born to 3 women in group 1 died from asphyxia during or shortly after delivery In all instances the diagnosis was made at autopsy There had been itching during 2 of the preg nancies one of which was also complicated by pyelitis proteinuria and elevated BP The mothers of the children born after uncomplicated preg nancies had itching with jaundice during other pregnancies Gestation varied from 35 to 45 weeks and the birth weights from ..20 to 950 g.

Three children (2 from group 1 and 1 from group who died in utero before delivery were born erated No pecific cause of death could be ablished There had been itching during of the pregnancies

Notes of meconium staining of the amniotic fluid considered a gn of fetal asphyxia (16) were found in 48 li ery records (Table V) in the absence of obstetric omplications or disease of the child The incidence was 9? (8 of 86) in children born after uncomplicated pregnancies and 27% (37 of 149) in those born at deliveries with itching. Among the latter there was no difference between children of women with recurrent and non-recurrent symptoms Factors which might increase the risk of asphyxia (infection during pregnancy proteinuria elevated BP premature delivery or low birth weight) were present in 15 of the 37 deliveries with itching, and in 4 of the 8 deliveries without.

Rickets was discovered at the age of 8 months in one child born after a pregnancy complicated by itching. Several other children had had medical care for reasons other than infectious disease and minor traumata but there was no concentration of any disease or anomaly to any special group

The women

Eleven women (12%) reported that a close relative had had itching during pregnancy (Table VI) The highest prevalence 18% was found among the women with recurrent itching (group 1)

At the follow-up the majority of the women later

Table V *Deliveries with meconium staining of the amniotic fluid*

	Itching at delivery		Itching ending before delivery		No itching	
Group	Total	With meconium staining	Total	With meconium staining	Total	With meconium staining
1	103	4			13	1
2	30	7	6	3	73	7
3	16	6	2	0	–	
Total	149	37	8	3	86	8

Table VI. *Itching and jaundice during pregnancy in relatives of the women investigated*

Group	Case no.	
1	17	Mother had itching during all 8 pregnancies
	25	Mother had itching during her only pregnancy
	26	Sister had severe itching during 1 of 2 pregnancies
	37	Sister had itching during her only pregnancy
	79	Sister had itching during her only pregnancy
	96	Daughter had itching during her only pregnancy
2	13	One of 2 sisters had itching during all 3 pregnancies
	47	Sister' daughter had itching during pregnancy
	80	Daughter of maternal uncle had itching during her only pregnancy
	86	Maternal aunt had jaundice during 1 of 5 pregnancies
3	32	Mother had itching during the first of 4 pregnancies
	94	Mother had itching during one or more of 3 pregnancies

viewed considered themselves to be in good health. Of the 89 women 69 have been admitted to hospital for reasons other than childbirth and complications during pregnancy. Apart from biliary tract disease the most common causes of admission have been benign disorders requiring surgical or gynecological treatment. Medical care for urinary tract disease was reported by 11 women 3 of whom (1 from each group) had suffered from recurrent urinary tract infection.

Nineteen women had been cholecystectomized because of gallstones, and 16 showed gallstones at cholecystography giving a minimal prevalence of cholelithiasis of 39% (Table VII). Twenty-one women (7 in group 1 21% 10 in group 2 27% 4 in group 3 22%) did not consent to a cholecystography. The prevalence of present or previous cholelithiasis was higher among women with recurrent than non-recurrent itching (59 and 24% respectively). The prevalence was 41% (28 of 68) in women below 50 years of age and 33% (7 of 21) in those above that age. Of the women who were unwilling to undergo X-ray examination 5 (3 in group 1) had symptoms suggesting gallbladder disease. These women had experienced epigastrial or upper right abdominal pain following ingestion of fats, eggs or raw fruits. One woman (group 2) was cholecystectomized 3 years before her period of itching and 4 women (group 1) were operated on between pregnancies with itching. The remaining 14 were cholecystectomized 6–17 years (median 12) after the first period of itching.

Twenty-seven women had tried oral contraceptives for periods ranging from a few months to 8 years (median 2.5 years). Ten of them were referred to group 1. None had experienced itching or jaundice during these periods.

DISCUSSION

Cholestasis of pregnancy may be difficult to differentiate from other conditions associated with itching and jaundice such as infectious hepatitis. Laboratory examinations including determination of the Au antigen and liver biopsy facilitate the diagnosis. In the present women the diagnosis of cholestasis of pregnancy was made mainly from the clinical picture. The recurrence of typical symptoms during successive pregnancies in the women of group 1 strongly indicates that they suffered from cholestasis of pregnancy. Retrospectively this diagnosis may be questioned in some women with non-recurrent symptoms. Thus it is remarkable for instance, that itching ended before delivery in 6 women in group 2. In the

Table VII *Prevalence of gallstones*

Group	No. of women	Cholecystectomized or abnormal cholecystography	Normal cholecystography	Cholecystography not performed: Symptoms of gallstones (Typical)	(Non-typical)
1	34	20	7	3	4
2	37	9	18	2	8
3	18	6	8	0	4
Total	89	35	33	5	16

remainder the clinical course was entirely compatible with cholestasis of pregnancy. When only one of several pregnancies is complicated by itching, the question of precipitating factors arises. In the present series the only factor found to be overrepresented in pregnancies with non-recurrent itching was twin births. Twins were born after 3 of the 36 full-term pregnancies complicated by itching in group . An overrepresentation of twin pregnancies in cholestasis of pregnancy has been observed earlier (13, 19). It is conceivable that a larger hormone load in such pregnancies may be of importance.

Infections, especially bacterial urinary tract infections, have been implicated as possible provoking and aggravating factors in jaundice of pregnancy (11, 13, 24, 27, 28, 31). However, such disease was not common among the present women. Urinary tract infection was diagnosed during 1 pregnancies, of which 9 were complicated by itching. It is usually not clear from the records whether itching occurred in temporal association with the infection. Furthermore, when the women were not pregnant they do not seem to have suffered from urinary tract disease unduly frequently, as far as can be ascertained from the interviews.

Disregarding the possibility that itching may have been caused by disease other than cholestasis in a few women, the incidence of cholestasis may calculated. During the time when the present ies (initially 100 women) was collected there ere approximately 3 000 deliveries at Malmö General Hospital, giving an approximate incidence of cholestasis of pregnancy of 3%. In two recent Swedish investigations (14, 3) the corresponding figures are 1.6 and 4%. The incidence of cholestatic itching is reported to vary from 0.27 to 14% in other countries (7, 15, 26). The last figure originates from Chile, thus corroborating the impression that cholestasis of pregnancy has a peculiar geographical distribution, being most often reported from Chile and the Scandinavian countries (1).

Among the present women, delivery tended to occur earlier in pregnancies with itching (40th week) than in uncomplicated pregnancies (41st week). The earliest median time for delivery (39th week) was found in complicated pregnancies of those women who had jaundice. This is in accordance with the figures from complicated deliveries in the previous study of women with jaundice in pregnancy (8). An unexpectedly short gestation has been observed in some cases of itching without jaundice (3, 32), but in a recent Swedish report (14) the time of delivery in 57 women with cholestatic itching was considered normal (median 40th week).

Other reports of women with itching due to cholestasis of pregnancy (6, 14, 18, 26) indicate that the children both of patients and normal controls have approximately the same birth weight. In the present series, children born after pregnancies with itching tended to have a lower median birth weight than those born after uncomplicated pregnancies, compatible with the shorter gestation period in the former.

Meconium staining of the amniotic fluid, considered to be a sign of fetal asphyxia, was found more often if itching had occurred at delivery (27%) than if no itching had occurred during pregnancy (9%). Additional complications known to cause asphyxia do not explain this discrepancy as they were present to an equal extent in pregnancies with and without itching. The present incidence of 27% is in accord with the figure of 22% for meconium staining of the amniotic fluid in a previous follow-up study among children whose mothers had had itching and/or jaundice at delivery (9). Fetal asphyxia was also described in a report of 9 cases of pruritus of pregnancy (3).

Among the present children there is nothing to indicate that mortality from causes other than asphyxia should be related to the mother's itching. The child with rickets is the only one in whom itching during the mother's pregnancy may be suspected to have caused impaired health. The mother had itching from the beginning of the 8th month until after delivery in the 39th week. Two other children in whom rickets was discovered at an early age, and whose mothers had jaundice as well as itching when pregnant, have been reported earlier (9, 21). It has been suggested that malabsorption of vitamin D in the mother due to cholestasis could predispose to a deficiency state in the child (9).

The high prevalence of cholelithiasis in women with recurrent cholestasis was the most striking finding of the present follow-up. The overall minimal prevalence in the present series was 39% (35 of 89). Although this may seem a high figure, the prevalence of gallbladder disease in two recent

Table VIII *Prevalence of gallstones (GS) in relation to number of full-term pregnancies and age at follow-up (mean and range)*

Group	No. of women with GS*	Age (y)		No. of full-term pregnancies	
		GS	No known GS	GS	N known GS
1	20/34	43 1 35–57	45.9 34–59	3.6 2–7	3.5 2–8
2	9/37	47 8 41 58	44.2 35–56	3.2 2–6	2.9 2–6
3	6/18	43.6 40–47	44 8 37–54	1	1
Total	35/89	44 4	44.8	3 1	2.6

GS/total no

autopsy studies from Malmö (17 29) in women of comparable age was 35 and 31% respectively There is, however a pronounced difference between the women with recurrent itching (group 1) and the other women (groups 2 and 3). In group 1 the minimal prevalence of gallstones was 59% (20 of 34) whereas the corresponding figure for groups 2 and 3 was 27% (15 of 55). As among the women with jaundice in pregnancy it is unlikely that the difference in prevalence of cholelithiasis between the present women with recurrent and non-recurrent itching depends on differences in parity or age (Table VIII).

The prevalence of gallbladder disease among the women with recurrent itching is in good agreement with that (16 of 28) found at a previous follow-up study of women with jaundice due to recurrent cholestasis of pregnancy (11) Combining the two series the minimal prevalence of gall bladder disease is 58% (36 of 62) in women who have suffered recurrent cholestasis of pregnancy By comparison when the two autopsy series are combined, the prevalence of gallbladder disease is 33% (104 of 313) This difference is statistically significant ($p<0.001$)

Most of the women considered themselves to be in good health at the follow-up and there were no indications of any severe liver disease in the histories Consistent with the general view that cholestasis of pregnancy is a benign disorder (12) it is then unlikely that any significant number of the women have suffered permanent liver damage of clinical importance as a result of itching during pregnancy

Cholestasis of pregnancy and cholestasis due to oral contraceptives are considered to be related disorders and occur sometimes in one and the same woman (1). However none of the women in the present series reacted with itching or jaundice to oral contraceptives This is in accordance with Rannevik et al. (23) who demonstrated that oral contraceptives only rarely cause significant disturbances of liver function in women who have suffered itching and/or jaundice in pregnancy

In a previous study (10) a familial occurrence of itching or jaundice in pregnancy was found in 7 of 16 women (44%) with typical recurrent cholestasis as against one of 25 women with non-recurrent jaundice. Among the present women 6 of 34 (18%) with recurrent itching knew of a relative with itching during pregnancy The mildness of the syndrome in the present women and their relatives may help to explain the lower figure. It seems reasonable that a less dramatic clinical picture would be less known outside the immediate family circle

It would then seem from the present follow-up study and from the follow-up study of jaundice in pregnancy (8 9 10 11) that women with recurrent cholestasis of pregnancy with itching as the most prominent symptom, and women with recurrent cholestasis with jaundice suffer from the same disorder and that the implications for the mothers and children are the same only differing in degree. Thus the hereditary predisposition and the tendency to early delivery were less pronounced in the former women than in the women with both itching and jaundice However both the prevalence of gallbladder disease in the women at follow-up and the incidence of asphyxia

at birth in their children were the same for all investigated women with recurrent cholestasis of pregnancy whether the symptoms had been itching and jaundice or itching alone

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LONG-TERM CONSERVATIVE THERAPY OF CHRONIC AV BLOCK

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Abstract Thirty-six of 98 patients with chronic advanced AV block have been treated conservatively by us, during the 5-year period 1966–70. Follow-up studies were made until Aug. 1973. When oral test doses of isoprenaline increased the heart rate without side effects, long-term isoprenaline abolished syncope in most patients both with normal and with widened QRS complexes. Eighteen patients died during the observation period, but death was in most cases unrelated to block. The study shows that conservative therapy may be used with fair success in selected patients.

Even if the indications for pacemaker therapy in chronic advanced atrioventricular (AV) block have been widened many patients are still treated medically (1). The results of permanent pacing have been extensively published but reports on long-term drug therapy are scarce. The purpose of the present report is to show the results of 5-year conservative therapy with follow-up in a regional hospital

PATIENTS

In the 5-year period 1966–70 98 patients with chronic symptomatic 2nd or 3rd degree heart block were admitted to our Department of Medicine Follow-up examinations were made until Aug 1973 for an average of 5 years, at least 2.5 years.

Thirty-six patients, 22 men and 14 women, and 56–92 years (mean 76, 11 patients <70, 11 70–80, 14 >80), were treated conservatively while 62 received permanent pacemakers. The reason for conservative treatment was mainly increased heart rate and clinical improvement after trial of oral isoprenaline therapy

Thirteen of the 36 patients had no relevant previous or associated diseases. Myocardial infarction had occurred previously in 5 patients, but in at least one of them AV block had been recorded earlier In the 5-year period advanced AV block developed during acute infarction in 105 patients, but persisted in 3 only (6).

Four patients had angina pectoris None of them had syncope in association with chest pain Ten patients had aortic valve disease which in all was judged to be only slight or moderate. Of the remaining 4 patients 2 had rheumatoid arthritis, 1 a history of rheumatic fever and 1 endomyocardial fibrosis.

One or more episodes of syncope had occurred in 22 of the patients and congestiv heart failure was present in 18. Ten patients had only insignificant symptoms slight dizziness, tiredness, etc

Third degree AV block was present in 28 patients (QRS above 0.12 sec in 17 and below in 11) and 2nd degree AV block in 8 (QRS above 0.12 in 2 and below in 6). The AV block was intermittent in 16 patients (QRS above 0.12 in 6 and below in 10) and persistent in 20.

ECG patterns are shown in Table 1 Syncope had occurred in more than half of the patients both with normal and widened QRS Syncope occurred equally frequently whether the block was intermittent or not.

The duration of AV block was unknown in 16 patients 9 of whom were admitted to hospital after their first syncope Among the 20 patients with know duration of heart block 4 had a short history of a few days up to 3 weeks. In the remaining 16 patients AV block had lasted for 6 months up to 16 years (mean 7.4 years).

TREATMENT

Short-acting isoprenaline was given sublingually to 10 patients 4–6 times or more a day during the observation period. The average daily dose was 60 mg (37.5–90). *Long-acting isoprenaline* was given to 16 patients twice a day The total daily dose varied from 60 to 40 mg. The response was first tested by counting the heart rate every 15 min or by continuous monitoring for 2 hours after oral short-acting isoprenaline first 7.5 mg and then, if necessary after increasing doses up to 30 mg. The i.v. isoprenaline test (9) was not used routinely as occasionally good i. heart rate response was not followed by response after sublingual doses. Ten patients received diuretics and 8 digitalis In addition to isoprenaline while 10 had such sparse symptoms that no therapy was given.

have been reported in up to nearly 25% (7). We have observed breakage of the electrode in about 10% of the paced patients often after several years. Development of unexplained congestive heart failure after pacemaker implantation has been reported (3).

Isoprenaline may also cause discomfort and complications such as tachycardia sweating and tremor which have been noted in 20–25% of cases (1). In our *long-term* treated patients unpleasant side-effects were not observed probably due to the rather low doses compared with many other studies.

How frequently is isoprenaline therapy sufficient? Among *all* of our 98 patients with advanced chronic AV block isoprenaline was attempted in 41. Fifteen of them had to be treated with pacemaker either because of no effect or because of side-effects especially ventricular or supraventricular arrhythmias.

In conclusion pacemaker therapy may offer the best results as regards long-term prognosis in chronic symptomatic advanced AV block. But it may not be feasible to treat all patients with pacemaker. The present study shows that long-term therapy with isoprenaline can be used with fair success and few side-effects if the heart rate increases and the clinical condition improves after relatively low oral doses.

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REMOVAL OF EXOGENOUS PLASMA TRIGLYCERIDES IN SPLANCHNIC VISCERA IN MAN DURING ANESTHESIA

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Abstract The role of the splanchnic viscera and the liver in removal of an artificial fat emulsion Intralipid® has been studied in anesthetized man Ten males and six females were studied. During operation for chole cystectomy catheters were placed into an artery the portal and the hepatic veins. During a constant i. in fusion of Intralipid® the concentration of the fat emulsion was determined by nephelometry in blood plasma sampled at intervals from these three vessels. Splanchnic plasma flow was estimated simultaneously During the infusion constant concentration of Intralipid was found in the blood. The splanchnic flow was not af fected by the infusion of the fat emulsion. The difference in mean arterio-portal concentration of Intralipid was 69±15 µmol/l at 20 and 31 min, corresponding to a removal of 5.3±1.2% of the arterial concentration ($p<0.001$) The amount of infused Intralipid® removed per minute was 35±8 µmol, corresponding to 25±6% of the infused quantity ($p<0.001$). No significant changes of mean concentration were observed between the portal and the hepatic eins.

In studies of the role of the splanchnic region in the turnover of triglycerides (TG) from the blood stream in man (4 26) the technique of hepatic vein catheterization has been used With this method the TG uptake and release both by the liver and the splanchnic viscera are estimated. However the idea has been put forward that part of the TG eliminated from the blood may be taken up by the splanchnic viscera (4). There are several reasons for this possibility It is well known that the omental adipose tissue is metabolically very active and several analyses of the lipolytic activity have demonstrated higher activity in omental adipose tissue than in subcutaneous fat (9 22, 23) Chylomicrons are believed to be removed both by liver and adipose tissue as intact particles (24 27 28) or after hydrolysis (15 19) Recent investigations have suggested that the liver is a minor site of direct incorporation of chylomicron (1 14).

Olivecrona and Belfrage (27) have demonstrated that n the carbohydrate-fed rat intact chylomicrons can be removed also in the small intestine In order to further evaluate the role of the splanchnic region in man with exclusion of the liver it is necessary to analyse portal blood

The i.v fat tolerance test (IVFTT) has recently been introduced as a simple test which determines the elimination rate from the blood stream of exogenous TG using the artificial fat emulsion Intralipid® (Vitrum Stockholm) (5 11). This test also significantly reflects endogenous plasma TG turnover (29). Little information has been available about the fate of the emulsion n man during its elimination. In a previous study we have demonstrated that 6% of the arterial concentration of Intralipid® was taken up by the human heart (10). The aim of the present study was to study whether Intralipid® which in several respects resembles chylomicrons (3 8 17) was taken up n the splanchnic region and in the liver

SUBJECTS DESIGN OF STUDY AND METHODS

Subjects

Two studies were performed: a pilot study of 5 male patients aged 37–58 (study I) and a second of 6 females aged 25–57 and 5 males aged 37–58 (study II)

The patients were admitted to the Department of Surgery for cholecystectomy because of gallstones and were studied after their informed consent. All patients were free from ymptoms and the surgery was elective. Furthermore none had symptoms suggesting metabolic disorder or were on medication expected to affect lipid metabolism. Fasting plasma TG and cholesterol were determined before hospitalization. Three males in study I and four males and one female in study II had

Table 1 *Clinical data of the patients in study II (mean±S.E.M ranges within parentheses)*

Pat. no	Sex	Age (y)	B wt (kg)	Weight/height index (kg/cm-100)	Peroperative plasma lipids: TG (mmol/l)	Peroperative plasma lipids: Cholesterol (mg/100 ml)	Plasma volume (l)
1	♀	34	80	1 15	0.92	186	2.80
2	♀	35	80	1.27	1.25	240	2.80
3	♂	38	80	0 96	2.07	13	2.95
4	♀	46	63	0.93	1 17	_59	2.60
5	♀	45	50	0 94	1 9?	_58	2.35
6	♀	57	70	1 17	42	263	2.50
7	♂	51	102	1.31	3.06	216	3 70
8	♂	47	81	1 16	3 16	266	2.95
9	♂	53	86	1 12	2 69	214	3 10
10	♀	25	54	0 79	0 72	211	2.30
11	♂	58	70	0 97	1 79	226	_95
Females		40±5 (25–57)	66±5 (50–80)	1.04±0 07 (0 79–1.27)	1 40±0.26 (0 72–2.42)	236±13 (186–263)	2.56±0 09 (2.35–_80)
Males		49±3 (38–58)	84±5 (70–102)	1 10±0 07 (0 96–1.31)	2.55±0.27 (1 79–3 16)	227±10 (213–?66)	3 13±0.15 (2.95–3 70)

fasting plasma TG concentration above 2 mmol/l The clinical data of the subjects in study II are summarized in Table I

Design of st dy

IVFTT was performed in all patients 1–4 days before operation. Patients in study II were g en an i injec tion of 5–8 μC ¹²⁵I-album concomitantly with the IVFTT t permit determination of the plasma volume and detection of possible shifts in plasma water concentration between arterial and venous blood

Study I was aimed only at detecting differences i arterio-portal concentration of exogenou plasma TG ing constant infusion of Intralipid® Arterial nd al blood was drawn simultaneously 5 times 12 31 min after the start of the inf sion and the concentration of the fat emulsion was determined 10 times on each sample

Study I initiat d st dy II designed to quantify the splanchnic uptake of e ogenou TG For this purpose the hepatic vein was catheterized and blood was sampled for estimation of hepatic blood flow concentration differences across the liver The design and consecut ve order of the different procedures in study II are given in F g. 1

Anesthesia

All patients were studied in the morning after overnight fast and premedication with atropine and pethidine (ACO Stockholm Sweden) The patients were anes-

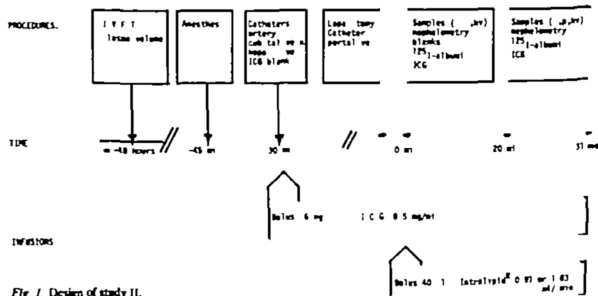


Fig 1 Design of study II.

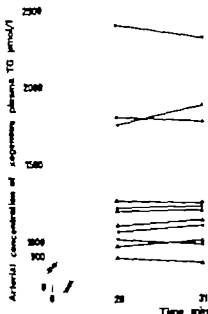


Fig. 2. Arterial concentrations of exogenous plasma TG in study II at 20 and 31 min. ● = Intralipid® infusion rate of 1.63 ml/min, ▲ = of 0.91 ml/min.

thetized with droperidol plus fentanyl (Dridol® and Leptanal® Leo Helsingborg, Sweden). They were intubated and ventilated by the Engström respirator (LKB Stockholm) with a mixture of 30% oxyge and 70% nitrous oxide during the study. When the patients had been anesthetized the studies were performed and after that the surgical procedure w s completed. Only saline was given during the study as a low drip infusion. All patients were discharged from the hospital without complications or blood transfusions.

Catheterizations

I all patients an arterial catheter was placed int the right brachial artery. In study II right hepatic vein was catheterized under fluoroscopic guidance from the right arm (4). Ten per cent Intralipid® and Indocyanine green (ICG) (Cardio-Green® Hynson Westcott & Dunning, Baltimore) were infused into peripheral veins by means of constant rate infusion pumps. The infusion rates of Intralipid® were either 1.63 or 0.91 ml/min after a bolus dose of 40 ml. The infusion rate was chosen on the basis of the fractional elimination rate k_2 calculated from the IVFTT performed before the operation. Patients with k_2 value below 4%/min received the slow infusion rate.

The portal vein was catheterized as described by Gonzalez Carballnes (16). A right-sided subcostal incision was made for the cholecystectomy. Ligamentum teres hepatis was easily identified and the rudiment of the umbilical vein was exposed. This occluded vein was incisioned and easily recanalized with blunt probes of 2–5 mm diameters. When the junction between the umbilical vein and the portal vein had been opened polyethylene catheter (PE 260) was inserted. The tip of the catheter was positioned in the left branch of the portal vein close to a umbilical junction to permit easy blood sampling. No heparin was given during the study. All catheters were kept patent either by slow saline drip or by intermittent flushing with saline.

Blood sampling

Initially 3 ml arterial blood was drawn for determination of fasting plasma TG and cholesterol. Furthermore blood was collected from the artery the portal vein and the hepatic vein (only study II) for estimation of blank values for the subsequent ICG and Intralipid® determinations. After the start of the Intralipid® infusion blood was sampled at ntervals, 15 ml blood was sampled simultaneously from the artery the portal and hepatic veins (only study II) for nephelometry ICG and ^{125}I-albumin determinations. Only dry plastic yringes were used without additives. Samples were draw at 1., 20 24 27 and 31 min in study I and at 20 and 31 min in study II. The portal catheter was not flushed until ll samples from the hepatic vein had been drawn. Altogether less than .50 ml blood was taken from each patient.

Intravenous fat toleranc test

IVFTT has previously been described in detail (14). One ml/kg b.wt. of 10% Intralipid® was given i. as pulse injection and blood samples were taken every 5 min during 40 min. The light scattering index (LSI) of the plasma samples diluted 1 100 was read in a Thorp micronephelometer and plotted in semilogarithmic ys tem, where a straight line was found. The lope of the line was calculated and called k_2 %/min. A standard curv was prepared from each bottle of I tralipid® for calculation of the TG concentration from the LSI obtained by nephelometry.

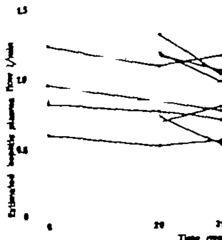


Fig. 3. Estimated hepatic plasma flow during constant infusion of Intralipid®.

Nephelometry

From each arterial portal and hepatic vein blood sample, 10 times 90 µl plasma was dil ted 1 100 in saline for replicate nephelometry determinations (30) All samples (up to 120 tubes) were then randomized and the technician reading the nephelometer LSI did not know the origin of the samples. The average reading for the fasting plasma sample from the artery the portal and the hepatic veins was subtracted as a blank from the respective sample reading. Means±S E.M of the LSI were calculated nd LSI was converted to µmol/l Intralipid® TG by means of the standard curve and an assumed mean molecular weight for Intralipid® TG of 878

Chemical determ nation

Serum TG and cholesterol before operation were determined by an AutoAnalyzer technique (2, 20) In tw pa tients (nos. 1 and) the plasma TG concentration w s determined in ten Folch chloroform-methanol extracts of arterial and portal blood samples respectively (7). The chloroform phase was washed and by subsequent AutoAnalyzer technique the TG concentration was determined on triplicates of each such extract (7 20).

As the Intralipid® content of the samples would have interfered with the spectrophotometric determination of ICG the samples were centrifuged in the ltracentrifuge at 36 000 rpm for 40 min. The ICG wa then determined on plasma in a Beckman pectrophotometer as suggested by Nielsen (25) The plasma volume w determined with ¹²⁵I-albumi (3.) F rthermore the ¹²⁵I-albumin concentration was determined in the plasma replicates from each sample in study II

C lculation

The calculation of the fractional removal rate k n he IVFTT hav previously been described (11) The lculation of the fractional removal rate of Intralipid® during the constant infusion was performed according to the formula

$$k_c = \frac{\text{infused Intralipid® µmol/min}}{A_{LSI} \times C \times PV}$$

where A_{LSI} is the mean arterial plasma LSI minus blank value C is a factor for the conversion of LSI to Intralipid TG (µmol/l) and PV is the plasma volume (l).

The blood volume on the day of operation was assumed to be the same as du ing the IVFTT A steady stat concentration of the fat emulsion in blood was found within 20 min of constant infusion (Fig. 2) The splanchnic blood flow was estimated by measuring ICG concentration in the artery and the hepatic vein as de scribed previously (6). Corrections due to a small but significant arterio-portal difference in ICG concentration will be presented and discussed below Of the hepatic blood flow 80 % wa assumed to come from the portal vei and 20 % from the hepatic artery

The elimination (E) of Intralipid® in the splanchnic region except the liver was calculated according to the formula

$$E = (A_{LSI} - P_{LSI}) \cdot C \cdot ESPF \cdot 0.8 \text{ µmol/min}$$

wher P_{LSI} is the mean portal plasma LSI minus blank lue and ESPF is the estimated splanchnic plasma flow (l/m)

RESULTS

The constant infusion of Intralipid® resulted in a constant concentration of fat particles in the blood stream as determined by nephelometry (Fig. 2). The fractional removal rate determined from the

ble II *Arterial (a) oncentration of exogenous plasma TG differences in arterio-portal (a-p) concentration and planchnic remo al of the infused Int alipid® (nephelometric determinations m an±S.E M)*

	20 min			31 min			70 min	
Pat. no.	a (µmol/l)	a-p (µmol/l)	% of a	(µmol/l)	a-p (µmol/l)	% of a	Removal (µmol/min)	% of infused I tralipid®
1	1 010±10	70±10***	7 9	960±10	60±10***	5 8	41	22
	1 040±10	70±20*	6 3	1 110±10	40±20*	3.6	–	
3	970±20	10±20ns	1 1	1 020±10	80±10*	8.3	7ns	7
4	1 810±10	150±20*	8 3	1 790±10	100±20***	5 7	60***	32
5	1 760±10	−10±20ns	−0.5	1 890±10	40±10*	2.	−8ns	−4
6	1 200±10	−30±20ns	− 3	1 220±10	10±20ns	0 7	−24ns	−23
7	870±10	10±20ns	1 7		–		13ns	1
8	1 100±10	80±10***	7 6	1 140±10	90±20***	7 7	56	54
9	1 270±10	70±20*	7 7	1 40±10	40±10*	1 6	54	52
10	2 410±10	−10±20ns	−0.7	320±10	130±30***	1 8	−16ns	−9
11	1 220±20	200±20***	16 7	1 240±10	250±20***	20.5	118***	64
Mean±S E.M.	1 330±140	55±21	4 9±1 7*	1 390±140	88±22***	5 8±1.8	30±14ns	20±9*
Mean	(20+31 min = 1)		5 3±1 ** (% of)		69±15	(µmol/l)	33±8*	(µmol/min)

ns $p>0.05$ * $p<0.05$ ** $p<0.01$ *** $p<0.001$ based on *t*-test of difference between two means.

preoperative IVFTT (k_2) was compared to the k_c value during the infusion in study II. The mean k_2 was 4.73±0.69 %/min and the mean k_c 6.03±0.45 %/min ($p>0.05$). The constant infusion of ICG in the patients of study II resulted in a constant concentration in arterial as well as hepatic vein blood. The calculated hepatic plasma flow was not significantly changed during the experimental period in this study (Fig. 3).

The pilot study

The LSI of portal blood plasma was significantly lower than the corresponding arterial values in 1 of the 5 calculated differences in the 5 patients. In two arterio-portal pairs a significant negative difference of concentration was found (5.3 and 6.8% of the arterial concentration). The difference in mean arterio-portal concentration (±S.E.M.) was 30±9 μmol/l ($p<0.01$) correspond ng to a removal of 1.51±0.67% ($p<0.05$) of the arterial Intralipid® concentration.

Study II

From the 11 patients in this group 1 pairs of arterio-portal samples were obtained at 20 and 31 min. In 16/21 pairs a significant positive difference in arterio-portal concentration of Intralipid® as determined by nephelometry was found. No significant negative differences in arterio-portal concentration were observed. No differences in mean arterio-portal or portal-hepatic venous concentration of [131]I-albumin were found. The mean arterial ICG concentration was 0.9? mg/l and the mean portal ICG concentration 0.89 mg/l ($p<0.01$).

The difference in mean arterio-portal concentration of Intralipid® TG was 55±21 μmol/l at ?0 min ($p<0.05$) and 84±22 μmol/l ($p<0.01$) at 31 min (mean±S.E.M.). The individual values for each subject are given in Table II. These differences in arterio-portal concentration corresponded to a reduction of 4.9±1.7% ($p<0.05$) of the arterial concentration at 20 min and 5.8±1.8% ($p<0.05$) at 31 min. In patients 1 and 2 the differences in arterio-portal TG concentration as determined by nephelometry were 70±20, 40±20, 70±10 and 60±10 μmol/l. The corresponding figures obtained by the chemical method were 9±7, 46±7, 70±13 and 33±10 μmol/l respectively.

The difference in mean concentration of exogenous TG between the portal and hepatic veins, −18±30 μmol/l at ?0 min and −17±19 μmol/l at 31 min, are not statistically significant. The amount of infused Intralipid® removed in the splanchnic region during steady state conditions was calculated. At ?0 min 30±14 μmol/min (S.E.M.) was removed corresponding to 20±9% ($p<0.05$) of the infused amount of Intralipid®. The corresponding figure at 31 min was 41±9 μmol/min, ?9±6% of the infused Intralipid® ($p<0.01$). There was no correlation between the two infusion rates and the absolute or percental removal of Intralipid® or between infusion rates and the amount removed per minute. The fractional removal rates as determined by k_2 and k_c, the serum TG and cholesterol concentrations and the weight/height index did not correlate significantly to the differences in absolute or percental arterio-portal concentration or the uptake per minute of exogenous plasma TG.

31 min

Removal (μmol/min)	% of infused Intralipid®
31 **	17
42**	41
39*	21
26	14
9**	9
54***	52
32**	31
32*	17
104**	56
41±9**	29±6

25±6 (% of infused Intralipid®)

DISCUSSION

Methodology

In this study the changes in concentration of Intralipid® were determined by nephelometry. As nephelometry is a method by which the light scattering of plasma is studied, it is essential to verify that changes in light scattering agree with changes in concentration of exogenous plasma TG. Nephelometry is especially sensitive in the recognition

of larger particles since an increase of the particle radius by a factor is equivalent to a LSI increase by at least the square of the factor (30) A reduction of Intralipid® particle size during the passage through the splanchnic region would thus present as a reduction of light scattering although the plasma TG concentration remained unchanged However in earlier studies it has been shown that there is an excellent agreement between nephelometry and chemical methods (21) In the present study a comparison was also made in two cases between nephelometry and chemical determination of plasma total TG after extraction with chloroform-methanol and washing of the chloroform phase The comparison indicates that the changes in nephelometric concentration between the artery and the portal vein reflected a decreased concentration of Intralipid® As discussed in detail in a previous study (10) the small differences in concentration observed make it mandatory to apply methods with high precision If a technique for separation of exogenous from endogenous TG involving too large a methodological error had been applied in the present study it is possible that statistically significant differences in concentration would not have been observed

The current finding of a significant arterio-portal difference of 0.03 mg ICG/l i.e on an average 3% of the arterial concentration deviates from the observations of Viallet et al (31) who reported non-significant differences in arterio-portal concentration of both ICG and bromsulphthalein. The extrahepatic elimination of ICG has been considered insignificant for practical purposes (12) but if differences in ICG concentration were to indicate a water shift, the differences in TG concentration would be largely influenced in the present study As no difference in arterio-portal concentration of ^{125}I-albumin was found this argues against such a mechanism and makes an extrahepatic ICG removal in gastrointestinal organs probable.

Splanchnic removal of exogenous plasma TG

Under the assumption that the nephelometric determinations of differences in Intralipid® concentration reflect a removal of the fat emulsion the following reflections may be made During the passage from the arterial system to the portal vein a reduction of the exogenous plasma TG concentration by means of 5.3% of the arterial concentration was observed In this study however no significant difference in concentration of exogenous plasma TG was found across the liver the grand mean being −3±3% The passage through the splanchnic region to the portal vein involves a number of tissues such as the gastrointestinal tract, the spleen and the omental adipose tissue The design of the study does not make it possible to conclude which tissues preferentially removed Intralipid® from the circulation However it has been indicated that the reticuloendothelial system does not take up Intralipid® to any great extent (13). This is supported by our finding that there was no significant uptake between the portal and hepatic veins

The liver is known to contain a substantial number of phagocytic Kupffer cells which therefore seem to be without major importance for the removal of Intralipid® under the conditions of our study Therefore it is possible that the spleen as well may be of minor importance Although several studies have demonstrated a chylomicron TG removal in the liver others have stressed the importance of adipose tissue rather than the liver in the metabolism of chylomicron TG (1 27). Felts (14) has even suggested that the liver does not take part at all in the direct metabolism of chyle TG

The amount of chylomicron TG removed in the liver by the carbohydrate-fed rat has been estimated to be about 1/3 of a lipid load the rest being mainly removed in the adipose tissue (27). Our present finding demonstrates how important it is to study the role of the various parts of the splanchnic and hepatic systems separately and is in agreement with studies showing little hepatic uptake of chylomicron TG Previous studies on the omental adipose tissue indicate that this tissue is very active in lipid metabolism and may possibly be responsible for the removal

The extraction of the Intralipid® emulsion in the myocardium has previously been studied (10) and a decrease of the exogenous plasma TG concentration by grand means of 6% was reported In the present study we have demonstrated that the Intralipid® emulsion may be taken up in the splanchnic region as well The data on estimated splanchnic plasma flow make it possible to calculate roughly the fraction of the infused Intralipid® that is taken up in the splanchnic region. Even if the estimated hepatic plasma flow is cor

rected to give 3% higher values since the portal ICG concentration was lower than the mean arterial concentration the figures for the uptake of Intralipid® in the splanchnic region are very little affected

In this study it was of interest to note that the infusion of the fat emulsion Intralipid® did not result in flow changes in the splanchnic region This is in agreement with earlier findings in dogs and in contrast to flow changes induced by other nutrients, such as amino acids which increase the splanchnic plasma flow considerably (18).

CONCLUSION

By measurements with nephelometry of differences in arterio-portal concentration of i v Intralipid® the role of the splanchnic region in removal of this fat emulsion has been studied in anesthetized patients during operation for cholecystectomy The present study suggests that exogenous plasma TG can be removed significantly on passage through the splanchnic viscera. No significant net removal of Intralipid® was observed across the liver The grand mean±S E M uptake across the splanchnic bed was 5.3±1.-% of the arterial concentration. This corresponded to a removal of -5±6% of the Intralipid® infused per minute

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THE EFFECT OF A LONG-ACTING GLUCAGON PREPARATION ON ENDOCRINE AND METABOLIC RESPONSES IN ACUTE MYOCARDIAL INFARCTION

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Abstract. In acute myocardial infarction (AMI) the availability and the insulin-dependent transportation of glucose can possibly influence the extent of myocardial damage and the incidence of arrhythmias. Glucose metabolism is affected by several endocrine changes in connection with an AMI. Some of these changes have been studied in 43 patients with an uncomplicated AMI. A slight hyperglycemia was seen, with an increased catecholamine excretion and growth hormone secretion but with no reduction in the basal insulin secretion. The treatment of 27 of the patients with a zinc-protamine-glucagon (ZPG) suspension, s.c. 8 mg t.i.d. changed the natural response to an AMI only through a slight increase in the plasma growth hormone and insulin levels. The clinical course was not affected by ZPG which was tolerated without side-effects. It is concluded that treatment with ZPG is not indicated in uncomplicated cases of AMI.

Acute myocardial infarction (AMI) is accompanied by several endocrine and metabolic changes usually in relation to the severity of the myocardial damage and the clinical course. These changes include an increased secretion of catecholamines (6 15 16 18 19 25 43), cortisol (6 43) growth hormone (6 25) and glucagon (24). Insulin secretion is depressed during the first days (6 25 51) and elevated during the 6th–14th days (25) after AMI. Glucose tolerance is impaired and hyperglycemia is a common finding (5 4). Circulating free fatty acids (FFA) are increased (6 16, 18 21 43 44).

These changes can influence the extent and severity of myocardial necrosis (7 31 37) and alter myocardial contractility in an acutely failing heart (1 51). In the ischemic myocardium glucose serves as primary substrate for energy production (7 40), and the rate of its uptake is dependent on the availability of insulin (34). Diminished insulin secretion together with increased catecholamine and probably also with increased growth hormone secretion, account for elevated FFA levels (1 40) which have often been observed to be associated with a high incidence of arrhythmias (16 1) even if some reports have not confirmed this association (38 40 44).

The treatment of AMI with glucose insulin and potassium (GIK) was suggested by Sodi-Pallares et al (46 48). Although clinical experiments with this regimen or its modifications have shown a reduction in acute mortality only in a few minor studies (11 33 41) and no effect in many larger series (4 17 36 49) the importance of further studies for the reevaluation of this kind of treatment has also recently been emphasized (1 8 11 17 28 3 47 49).

Glucagon activates glucose production from glycogen in the liver (10 52) and it may be responsible for the hyperglycemia following AMI (24). It is also a potent stimulus to insulin release (3 10 50). Glucagon is used in the treatment of cardiogenic shock and intractable heart failure (8 13 27) because of its positive cardiac inotropic effect, and also because of the possible beneficial metabolic actions, as the GIK regimen also used experimentally (2, 28 51). Even if large doses of glucagon increase myocardial oxygen consumption and may enlarge the size of the infarct (31) there is a sufficient increase in myocardial blood flow to meet the growing myocardial demands (14 20 29 37). The inotropic effect is usually attained without any increase in the incidence of arrhythmias (13 39) and with facilitated atrioventricular conduction (39).

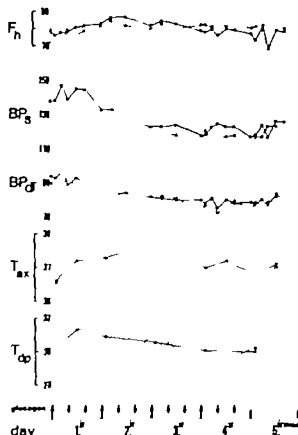


Fig 1 Mean heart rate (*F*) ystolic (*BP*) and diastolic BP (*BP*), and axillary (T_{ax}) and peripheral skin temperature (T_{dp}) of 22 glucagon-treated (—) and 11 control patient (-) The arrow show the times of injection

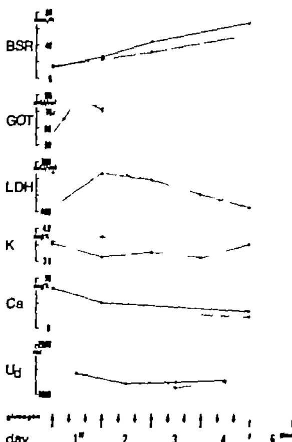


Fig 2 Mean blood sedimentation reaction (*BSR*) serum GOT and LDH enzymes, plasma potassium (*K*) serum calcium (*Ca*) and daily urine volume (*U*) of 22 glucagon-treated (—) and 11 control patients (-)

This study was undertaken in order to investigate the effect of glucagon administration on glucose production and insulin release in patients with uncomplicated *AMI* Concomitant excretion of catecholamines and growth hormone secretion was also studied in AMI patients with and without glucagon treatment The preparation used was a zinc-protamine-glucagon suspension (ZPG) with prolonged action (3 50) given subcutaneously 8 mg t.i d

MATERIAL AND METHODS

Patients and general measures All of the 43 male patients had definite AMI. Diabetic patients on insulin patients with serum creatinine values above 1.5 mg/ 100 ml and patients with uncontrollable arrhythmias or severe left heart failure on admission were not included. The patient were treated in the CCU of Turku University Hospital, which they had entered not earlier than 4 hours before the experiment started at 7 a.m The median time which had elapsed since the onset of typical chest pain was 17 hours for the 11 patients in the control group and 23 hours for the 22 patients in the glucagon group The means ± S D of age and weight these groups were 51 9±9 0 and 52 2±7.0 years and 68.5±8.3 and 74 7±11.2 kg respectively Two smaller groups of 5 patients each one control group and another treated with glucagon injections at 8-hour intervals, were followed closely with blood samplings every 2 hour for 24 hours. With the others the xperiment was continued for 4 days with glucagon injections at 8 m 4 p.m. and midnight. The sampling procedure is presented in Figs. 1 3 No placebo injections were used. An I. drip of 0 9% NaCl was sed instead of glucose infusion for the first 1–2 days. The average amount of inf sions was 780 m³ during the 1st day and all liquids given totalled 1510 cm³ The use of diuretics wa restricted to the absolute minimum The patient were allowed to take light meals at 11 a.m. and 4 p m Unrestricted sitting was on an average begun on the 3rd day and standing was allowed on the 5th day

Glucagon Subcutaneous injections of 8 mg ZPG were administered This preparation of slow-acting

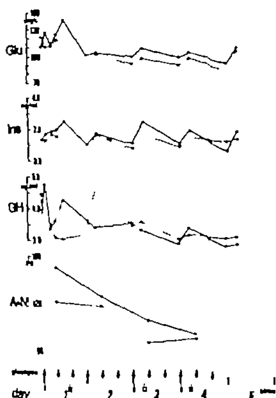


Fig. 3. Mean blood glucose (*Glu*), plasma immunoreactive insulin (*Ins*), growth hormone (*GH*) and daily urinary excretion of adrenaline and noradrenaline (*A+N*) of 22 glucagon-treated (—) and 11 control patients ( ).

glucagon (3 59) was manufactured and supplied by the Novo Industri A/S Copenhagen.

Clinical measurements. Heart rate (cardiotachometer), BP (cuff method), axillary and skin (dorsal toe) temperature were measured by standard methods of the CCU. Normal reference values were for GOT <19 mU/ml, LDH <240 mU/ml plasma potassium 3.3–4.8 mg/100 ml, serum calcium 8.8–10.8 mg/100 ml, blood glucose (fasting) 60–90 mg/100 ml and 24-hour urine adrenaline+noradrenaline <100 µg. Urine catecholamines were determined fluorimetrically by Pekkarinen method as described by Manninen (30).

Plasma insulin and growth hormone. Blood samples, drawn into heparinized tubes, were centrifuged and plasma was stored at −20°C until analyses. Plasma insulin and growth hormone were determined by the immunosorbent radioimmunoassay method (54). The standard preparation for insulin was human insulin WHO 166/304 and for growth hormone NIH-GH-HSL 394.

RESULTS

Clinical events. No deaths occurred either in the control group of 11 patients or in the glucagon group of — patients. No cases of severe cardiogenic shock, severe heart failure heart arrest or ventricular tachycardias were encountered. The numbers of patients with special therapeutic measures in the control and glucagon groups were as follows: lidocaine infusions 3 and 7, digitalis and 6, endocardial pacing 0 and patients respectively. No differences were seen in the number of injections of pethidine or pentazocine, or in attacks of vomiting.

Clinical measurement. The mean values of clinical measurements and of serum determinations are presented in Figs. 1–. BPs, particularly systolic and pulse pressure were non-significantly higher during the first day on glucagon. The peripheral skin temperature was higher ($p<0.05$) in the glucagon than in the control group. The lower plasma potassium and the higher serum calcium in the glucagon group than in the control group were not significant findings.

Endocrine changes (Fig. 3). A slight hyperglycemia as a response to the AMI on the morning of the first day of the experiment was found prior to the treatment in 8 of the 11 controls and in 16 of the 22 patients treated with glucagon. The mean blood glucose level of the patients treated with glucagon was slightly but not significantly higher than that of the controls. A fluctuation of blood glucose level was found in connection with the meals.

Plasma insulin levels followed the changes in blood glucose. There was no difference at any time in the study between the two groups and the fasting plasma insulin values were within normal limits. In the minor groups studied at -hour intervals during the first day (Fig. 4) the plasma insulin level was significantly higher at 10 a.m. and 2 p.m. in the glucagon group than in the control group.

Plasma growth hormone levels varied greatly in individual patients with high secretion peaks as seen in Fig. 4. In the minor groups significant increase in growth hormone levels was found 4 hours after the first glucagon injection. In only 3 of the 10 patients was a sleeping peak found. The growth hormone levels were quite similar in the major glucagon and control groups on the 3rd day of the study and afterwards.

The total daily urinary adrenaline and noradrenaline excretion was not significantly higher in the groups treated with glucagon than in the control groups. As expected a continuous and

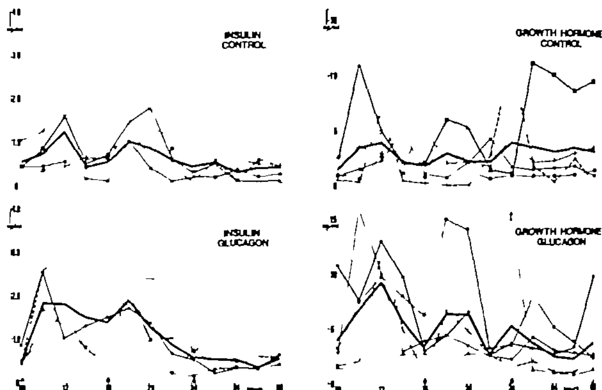


Fig 4 Individual levels of plasma immunoreactive insulin and growth hormone in 5 glucagon-treated and 5 control patient with AMI during the first 24 hours of the experiment. Mean levels are shown by the thick lines, the times for glucagon injections by the arrows.

significant decrease of the 4-hour catecholamine excretion was found in both major groups

The results were also examined in the light of e individual levels found Thus the 22 patients ated with glucagon were divided into two subgroups of 11 patients each according to higher or lower values during the first day of the study for urinar excretion of catecholamines mean levels of blood glucose plasma insulin or growth hormone and serum GOT The comparison of the endocrine findings in these two subgroups is shown in Table I The patients with a higher blood glucose tended to have a higher catecholamine excretion and a higher plasma insulin level but not a higher growth hormone level When the material was divided according to the insulin or growth hormone levels no differences in the other hormonal parameters were found.

DISCUSSION

The metabolic and endocrine effects of glucagon were studied in patients with an uncomplicated AMI

The changes we found in connection with the AMI itself were similar to those previously reported (see Introduction) e g. hyperglycemia increased levels of growth hormone and an increased excretion of catecholamines These changes were most pronounced during the first 4-hour period of the study As the median time from the onset of pain to the start of the experiment was 17 hours for the controls and 23 hours for those under glucagon treatment the peak of rapidly ensuring reactions had in some cases possibly been passed. The catecholamine secretion increases already during the first few hours following an AMI (15 53), as does hyperglycemia due to catecholamines and cortisol secretion (25). No depression in basic insulin secretion was found in our study This was not surprising, since a marked depression has previously been found in AMI, complicated by heart failure or cardiogenic shock (51) or in chronic circulatory failure (12 45)

When the endocrine response to the AMI was examined by checking up the plasma levels at 2 hour intervals during the first 4 hours in 10 patients the insulin secretion was found to be increased in a normal way after relatively light

Table I. *Endocrine findings in the high and low value halfgroups of glucagon-treated patients (means ± S.E.M.)*

GH = growth hormone, A+N = adrenaline and noradrenaline

Basis for division	GOT (mU/ml)	Glucose (mg/100 ml)	Insulin (ng/ml)	GH (g/ml)	A+N (μg)
GOT ≷ 70.0					
High	101.2 ± 7.41	7.27±0.30	1.78±0.33	3.14±0.40	168.6±48.5
Low	36.6 ± 8.11	6.27±0.30	1.88±0.56	3.56±1.21	184.5±43.4
Glucose ≷ 6.60					
High	7.27± 0.30	8.13±0.77	2.12±0.47	3.10±0.77	208.0±45.0
Low	6.27± 0.30	5.92±0.15	1.38±0.24	3.73±0.94	125.8±26.3
Insulin ≷ 1.75					
High	1.78± 0.33	6.57±0.37	2.53±0.35	3.39±[illegible].99	198.3±58.9
Low	1.88± 0.56	7.59±1.00	1.01±0.17	3.88±0.95	193.0±33.3
GH ≷ [illegible].35					
High	3.14± 0.40	6.84±0.36	1.66±0.27	5.19±0.94	173.0±34.7
Low	3.56± 1.21	7.21±0.91	1.80±0.49	1.72±0.19	179.4±48.2
A+N ≷ 120.0					
High	168.6 ±48.5	7.71±0.79	1.66±0.39	3.65±0.94	261.4±34.6
Low	184.5 ±43.4	6.34±0.25	1.84±0.23	3.20±0.79	77.3± 8.3

meals. A similar observation was made concerning the secretion of growth hormone, reflecting a paradoxical elevation in the postabsorptive state (9) and possibly also the additional exertion of eating in bed. A strong sleeping peak in growth hormone was seen during the hours before midnight in only 3 of 10 patients. A lack of or disturbed sleep in the CCU was found in connection with the absence of sleeping peak in most of the others.

Plasma levels of endogenous glucagon were not determined in this study. Secretion was probably increased in patients with elevated blood glucose levels as Lanindo et al. (24) found in their series of AMI patients. In our series the fasting blood glucose was over 90 mg/100 ml in 8 of 11 patients in the control group and in 16 of 22 patients in the group treated subsequently with glucagon. The "stress" hormones, catecholamines, cortisol and growth hormone, also promote hyperglycemia by gluconeogenesis and glycogenolysis, and the relative contribution of these hormones is largely unknown. Endogenous secretion of glucagon is, however, diminutive in comparison with the concentrations produced by the usual therapeutic administration of glucagon as a single i.v. dose of 5 mg or 2–4 mg/h as an infusion. According to Unger (57) the concentrations produced by cardiologists are probably 10 000 times the highest levels of endogenous glucagon, which were about 700 pg/ml in the series of Lanindo et al. (24).

We used a relatively small dosage of glucagon 24 mg/24 h, administered subcutaneously as a suspension in which the crystals are a combination of zinc, protamine and glucagon. Tarding et al. (50) found in mongrel dogs weighing 4–30 kg, that this suspension in a dosage of 1 mg caused a gradual increase in plasma glucose from about 170 to 150 mg/100 ml 1 hour after the injection. The concentration remained at this level for the rest of the experimental period, which was 6 hours. The FFA concentration declined to about 50% of the fasting level and returned slowly to the initial level. A serum insulin concentration of about twice the initial values was observed 1 hour after ZPG injection and maintained for at least 5 hours. Assan and Delaunay (3) tested the same ZPG preparation in man as a single s.c. injection of 10 μg/kg. This very small dosage yielded a plasma glucagon level of 500–1 000 pg/ml and a blood glucose level of 160 mg/100 ml in three subjects. The effects returned to the basal level within 3 hours and were considered to be not sustained enough to be of clinical importance. Kuhn (22) used 5–10 mg s.c. injections of ZPG in 11 patients with heart failure. The blood glucose level was found to be increased 92% after 3 hours and 43% after 6 hours. FFA decreased respectively to 35% and 29% of the initial values. In another series of 10 patients Kuhn et al. (23) found a significant increase of 141% in blood glucose 60 min after a 5 mg injection of ZPG.

I the present study no si   of

blood glucose was achieved with ZPG in the 8 mg t i d dosage An explanation might be the co-existent hormonal changes due to AMI The increase in plasma insulin concentration was significant in the small group of five patients 2 hours after the single injection of ZPG but not in the series of 22 patients treated with ZPG for 4 days After the cessation of ZPG treatment no individual cases of hypoglycemia or hypoinsulinemia were seen on the following day Kühn (22) reported severe hypoglycemic episodes in some patients possibly due to glycogen depletion in the liver after long-term treatment with glucagon

The positive inotropic response of the heart on glucagon treatment was also statistically non-significant in our study when evaluated with heart rate and blood pressure as parameters Glick (13) has suggested that if all the glucagon receptors of the heart are occupied by endogenous glucagon any additional glucagon administered by injection would have little or no effect. The concomitant activation of adenyl cyclase by catecholamines may also mask the action of glucagon in the heart. Levey and Epstein (26) found that the combined maximal effects of noradrenaline and glucagon on adenyl cyclase in cat heart did not exceed the effect of noradrenaline on its own. A combination of isoproterenol and glucagon has however been found to produce a greater effect on cardiac output in patients than either drug alone (42)

To conclude it can be stated that in patients AMI a larger or more frequent dosage of PG must be used than that administered by us if marked changes in the inotropic state of the heart or in the metabolic or endocrine response due to the AMI are desired

The usefulness of endogenous or exogenous hyperglycemia in AMI is still an open question as also is the importance of insulin administration. Glycolytic activity is near maximal already during normoglycemia and the possibilities of enhancing anaerobic glycolysis with insulin are limited (8 34) Brachfeld (7) also states that an augmented anaerobic glycolysis is not always desirable since the increase in intracellular lactate and other acidic end products will result in a dangerous drop in intracellular pH Most et al (35) have recently demonstrated that glucose extraction of ischemic human myocardium did not rise with increased arterial glucose concentration.

Our findings suggest that in uncomplicated AMI there is no need for treatment with either glucose insulin or a combination of them if the patients are allowed to take regular meals The condition of a patient with AMI complicated by cardiogenic shock or severe left heart failure may however be favourably influenced by the well tolerated injections of ZPG (22 23) with glucagon (13 27) or GIK infusions (2 28) In treatment with i.v infusions the rate and osmolality of infusions may be more important than the constituents of the solutions. This may also explain the varying results reported with various modifications of the Sodi-Pallares regimen (7) When an acute left heart failure in AMI is accompanied by reoccurring arrhythmias a functional improvement can be attained with glucagon without any increase in the incidence of dysrhythmias (13 39) Treatment with i v glucagon infusion is preferred in critically ill AMI patients but in acute exacerbations of chronic heart failure subcutaneous injections of a long-acting glucagon preparation may be both a practical and effective method of treatment (22).

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ACID–BASE PATTERN OF CEREBROSPINAL FLUID AND ARTERIAL BLOOD IN BACTERIAL MENINGITIS AND IN ENCEPHALITIS

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Abstract The acid–base status of cerebrospinal fluid (CSF) and arterial blood has been investigated in sequential samples from 25 patients with bacterial meningitis and 8 with acute encephalitis. Patients with bacterial meningitis hyperventilated spontaneously throughout the first week to median values for arterial carbon dioxide tension of about 30 mmHg. The pH in CSF was less than 7.30 in 17 cases, within the normal range in 5 and above 7.34 in 7 On admission the following CSF median values were observed. lactate=9 mmol/l hydrogen carbonate=14 mmol/l and pH=7.23 The concentration of lactate and pH in CSF approached normal values within week. Patients with acute encephalitis also hyperventilated but pH values in CSF were close to normal. Only severe cases of encephalitis had elevated lactate concentrations in the CSF Even in the presence of spontaneous hypocapnia artificial hyperventilation may be beneficial in cases where arterial carbon dioxide tension rises without simultaneous clinical improvement.

Bacterial meningitis and acute encephalitis are serious diseases with a definite lethality Bacterial meningitis can be treated with antibiotics. Additional symptomatic" therapy to maintain adequate ventilation and circulation has often to be applied. In patients with encephalitis this symptomatic therapy may constitute a main remedy

This study was part of a pathophysiological investigation in patients with bacterial meningitis and with encephalitis A report on cerebral circulation and metabolism has been published previously (12). The present study comprises the acid–base status in cerebrospinal fluid (CSF) and arterial blood (aB). The aim of both studies has been to obtain a better basis for a symptomatic treat of patients with bacterial meningitis and with encephalitis.

PATIENTS

The study comprises 33 patients admitted to the Department of Infectious Diseases Blegdam Hospital Copenhagen during March 1971 Jan 1972 Twenty-five patients had bacterial meningitis and eight had acute encephalitis.

Ten patients aged 18–28, had *meningococcal* meningitis. They all survived, and only one had residual damage (facial palsy) Artificial ventilation was not applied in these patients.

Nine patients, aged 46–77 had *pneumococcal* meningitis. Eight were critically ill on admission and three of them died one from ulcerative endocarditis one from obstruction by secretions the airways and one unexpectedly without definite explanation One patient had severe residual cerebral damage Three of the patients were artifically ventilated for 1–4 days Five patients—including the three latter—recovered without sequelae

One patient had meningitis caused by *Streptococcus pyogenes group A* He had severely impaired cerebral function but recovered completely A 5-year-old boy with *Hemophilus influenzae* meningitis had convulsions and respiratory arrest 9 hours after admission Despite artificial ventilation he remained brain-dead Cardiac action ceased 3 days later A 48-year-old man with a chronic suppurative otitis had *Proteus mirabilis* meningitis He recovered and was operated on for otitis 12 days after admission, but 14 days later he presented signs of brain abscess and died few days later Three patients had unquestionably purulent meningitis, but *no bacteria* were demonstrated One with lymphatic leukemia and bulbar symptoms died from pneumonia after 33 days One had destruction of the sella and pituitary insufficiency requiring substitution therapy After 6 months his cerebral function had recovered fairly well The third patient who had hypogammaglobulinemia, recovered completely from the infection.

Eight patients had acute encephalitis of unknown etiology Two patients had increasing antibody titers against herpes simplex virus, but brain biopsy not performed. Two patients had rather of the disease with headache, drow

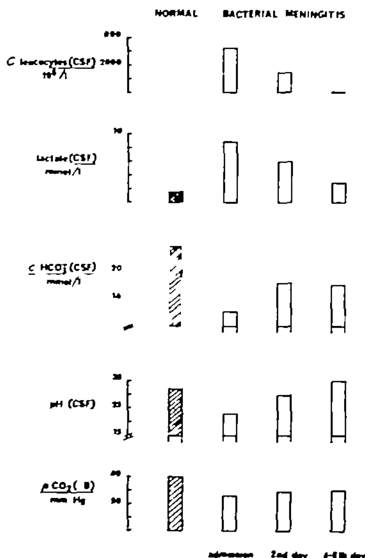


Fig. 1. Median values of some acid-base variables in 25 patients with bacterial *meningitis as compared to normal values.* c = concentration, C = number concentration.

fusion. A single case was moderately severe with marked confusion and agitation. Five cases were very severe with coma, generalized epileptic seizures or attacks of decerebrate rigidity; in four of them artificial ventilation was necessary. Seven of eight patients recovered practically completely, whereas one had repetitive epileptic seizures progressing to brain death.

METHODS

The 25 patients with bacterial meningitis were investigated within 24 hours after admission and institution of antibiotic treatment. Twenty patients were reinvestigated on the second day and 14 had a third investigation on the 3rd–8th day after admission. The eight patients with encephalitis were investigated 1–3 times within the first 16 days after onset of symptoms.

Samples of aB and CSF were drawn anaerobically in glass syringes. From 16 patients, in whom cerebral blood flow was studied simultaneously (12), CSF was obtained by cisternal puncture, whereas lumbar punctures were made in the others.

The following quantities were measured by means of equipment from RADIOMETER, Copenhagen. Carbon dioxide tension in aB pCO_2(aB) and in CSF pCO_2(CSF), was estimated using Severinghaus pCO_2 electrode type E5036/D616. Oxygen tension in aB pO_2(aB) was estimated using a Clark oxygen electrode type E5046/D616. The pH(CSF) and pH(aB) were assayed with an Astrup thermostated microelectrode unit and a pH meter (PHM 25) with a gas monitor (PHA 927). The pH(CSF) was estimated as described by Leusen (6). The standardized concentration of hydrogen carbonate in plasma, c(st)HCO_3^- (P) was assayed in fully oxygenated blood equilibrated at pCO_2=40 mmHg (4.5 kilo Pascal (kP)). The $cHCO_3^-$ (CSF) was determined at pH 7.4 after addition of a measured excess of HCl using titration assembly (TTA 31, ABU 11 and TTT1).

Concentrations of lactate and pyruvate in CSF clactate(CSF) and cpyruvate(CSF) were estimated by enzymatic methods (1). For these assays CSF was mixed

with an equal volume of ice-cold perchloric acid (c = 0.6 mol/l) immediately after sampling. A Zeiss PQM II spectrophotometer was used.

All assays were made in duplicate. The standard deviation of the mean of a duplicate determination (s) was calculated from the deviation of the two single values (No. of duplicates = 20). The results were: pH(CSF) and pH(aB) s = 0.005, pCO_2(CSF and aB) s = 1.0 mmHg (0.13 kP), pO_2(aB) = 2 mmHg (0.26 kP), $c$lactate(CSF) = 0.07 mmol/l, $c$pyruvate(CSF) s = 0.005 mmol/l, $cHCO^-$ (CSF) s = 1.15 mmol/l, c(st)HCO^- (P) s 1.3 mmol/l. pH, pCO and pO_2 were determined at 37°C, but corrected to the rectal temperature (4, 16).

RESULTS

Meningitis. The 25 patients with bacterial meningitis are considered as one group in Fig. 1 which gives the median values. The material is separated according to etiology in Table I showing median values and ranges.

On admission the leucocyte number concentration of CSF and $c$lactate(CSF) were increased to median values of $3\,200 \times 10^6$/l and 9 mmol/l respectively. The $cHCO_3^-$ (CSF) was reduced to 14 mmol/l, and the pH(CSF) to 7.23. The $c$pyruvate was increased, but considerably less than the $c$lactate (CSF), so that the lactate/pyruvate ratio was ~3 times the normal value.

During the first week both the leucocyte number concentration of the CSF and $c$lactate(CSF) decreased to median values of 85×10^6/l and 2.85 mmol/l, respectively. The $cHCO_3^-$ (CSF) increased already on the second day to 18.3 mmol/l, but remained subnormal throughout the study. The pH(CSF) gradually increased to approach the normal level within the first week.

Analysis of the aB on admission showed a hyperventilation with median values for pCO_2(aB) of 26 mmHg, for pH(aB) of 7.46 (normal range 7.35–7.42), and for c(st)HCO_3^- (P) of 21.7 mmol/l (normal range 22.0–26.0 mmol/l). Six patients had subnormal pO_2(aB) values below 80 mmHg, the lowest value being 47 mmHg. None of them had a meningococcal infection.

The hyperventilation continued throughout the study with median pCO values below 30 mmHg. The c(st)HCO_3 (P) was rapidly normalized and the pH(aB) remained slightly elevated. None of the patients who had normal pO_2(aB) values on admission showed subnormal values later on. Five of the six patients with subnormal pO_2(aB) values on admission also had values below 80 mmHg later on.

A survey of the results from the individual patients showed that a prolonged clinical course coincided with prolonged CSF abnormalities as regards pleocytosis as well as lactate(CSF). The CSF findings were of no value in predicting the outcome of the infection.

Only minor differences in the CSF data distinguished the three main categories of bacterial meningitis (Table II). Patients with pneumococcal meningitis tended to have a more long-lasting elevation of lactate(CSF) than those with meningococcal meningitis, the latter also having a more favorable clinical course.

Encephalitis. As shown in Table II the lactate(CSF) was hardly increased above normal in mild cases. In the severe cases with decerebrate rigidity or convulsions lactate(CSF) was moderately increased to ~6 mmol/l. The brain-dead patient had a $c$lactate(CSF) elevation to 13.5 mmol/l. The $c$pyruvate(CSF) and lactate/pyruvate [illegible] the CSF were moderately elevated in th[illegible] of cases. The $cHCO_3$ (CSF) was close to [illegible] in mild cases but reduced to a varying deg[illegible] severe cases. The pH(CSF) values were [illegible] normal with two exceptions. The brain-dead patient had marked acidosis. In case 6 the pH was clearly above normal.

Analysis of aB showed spontaneous hyperventilation with varying degree of pCO_2(aB) reduction, elevation of pH and usually normal pO_2(aB).

DISCUSSION

The typical finding in bacterial meningitis was a marked CSF lactacidosis and spontaneous hyperventilation in the acute stage. The CSF lactacidosis tended to subside rapidly whereas the hyperventilation continued. These findings were in agreement with previous studies by Montani and Perret (9) and by Blayo et al. ( ).

Patients with acute encephalitis had increased CSF lactate concentrations only in severe cases whereas almost all patients hyperventilated. We found no report on similar observations in patients with encephalitis.

These findings raise two questions: 1) What is the cause of the increased lactate concentration in the CSF in meningitis and in encephalitis. 2) What is the cause of the sustained spontaneous hyperventilation in these patients.

Table I *Characteristic findings at different times in patients with bacterial meningitis of different etiologies*

Both lumbar and cisternal CSF samples are included. Values of pH(CSF) and pCO_2(aB) from patients artificially ventilated are not included, except for cisternal CSF samples obtained during measurement of cerebral blood flow during artificial ventilation at pCO_2(aB) close to the level during spontaneous respiration
c=concentration C=number concentration, $\bar{x}$ = mean $s(x)$=S D of x
Median values and ranges. No. of investigation given within parentheses

	Normal values ($\bar{x}\pm2s(x)$)	Day of admission: Meningococcus M (n)	Day of admission: Pneumococcus M (n)	Day of admission: Other M (n)	Second day: Meningococcus M (n)	Second day: Pneumococcus M (n)	Second day: Other M (n)
leucocytes(CSF) (10^6/l)	0	2 91 (9) 513–33 000	4 850 (9) 37–8 064	4 000 (5) 766–18 500	1 179 (8) 14–4 140	1 640 (7) 80–3 000	1 6_ (4) 16–4 290
lactate(CSF) (mmol/l)	1 54 0.97 16	8 79 (10) 6.03–21.20	8 35 (9) 3.85–40.30	11 45 (6) 4 40–28 70	4 11 (8) _4 13.80	7.26 (7) 4 14–1_70	5 49 (5) 4.54–17.60
HCO_3^- (CSF) (mmol/l)	_3 6 1 8–25 4	16.8 (8) 7 6–18 6	15 0 (8) 9 7–20.0	13 0 (5) 11_ 20 5	20.5 (8) 13.8–2_6	14 4 (7) 10.8–19.8	17 4 (5) 13 4–22.3
pH(CSF)	7 3	7.266 (7) 6 967–7.383	7.218 (7) 6.948–7.360	7 168 (3) 7 157–7 297	7.299 (8) 7._31–7.340	7 _26 (5) 7 100–7.368	7.357 (5) 7 158–7 40
pCO_2(aB) (mmHg)	40	4 (9) 19–37	27 (8) 4–33	23 (5) 16–41	31 (8) 24–36	26 (5) 23–38	27 (5) 23–29

Table II *Cerebrospinal fluid and arterial blood quantities in patients with acute encephalitis*

No.	Day after onset	Clinical condition	Artificial ventilation (d after onset)	Cerebrospinal fluid: cleucocytes (10^6/l)	clactate (mmol/l)	cpyruvate (mmol/l)	clactate/cpyruvate ratio	HCO_3^- (mmol/l)	pH	pCO_2 (mmHg)
2	10	Mild		88	1 17	0.173	10 0	2_1	–	
7	4	Mild		15		–	–	–		–
	6	Mild		5	13	0 129	16.5	–		
	8	Mild			1.57	0 097	16_	23 0	7.346	40
4	3	Moderately severe		33	2 00	0.115	17 4	–	–	
	10	Mild		0	1 93	0.110	17.5	25.3	7.323	44
3	11	Severe		78	–	–	–	–	–	
	16	Severe	16–38	1	3 31	0 190	17 4	15 9	–	
5	4	Severe		104	4 40	0.154	28.5	–	–	–
	5	Severe		84	3 41	0.177	19 8	15 6		–
	9	Severe	5–10	136	6 05	0 479	12.7	17 7	–	–
6	2	Severe		15	2.69	0 211	12.7	4.8	7 423	40
	3	Severe		23	–	–	–	–	–	
	6	Severe	3– 7	–	5 02	0.375	13 4	–		
8	5	Severe		4	3 60	0 _37	15.2	21 6	–	–
	9	Mild		59	1 99	0 106	18.2	26.5	7.338	41
	11	Mild		56	1 61	0.09	17 7	22.6	7.291	47
1	13	Brain-dead	13–19		13.50	0.403	31.2	10 3	6.956	58

ourth–eighth day

Meningo-coccus M (n)	Pneumo-coccus M (n)	Other M (n)
48 (4)	31 (5)	1 020
0–154	21–1 408	32–1 856
06 (4)	4 64 (6)	5 05 (4)
1 40–4.76	2.30–8.46	2.76–9 04
23 8 (4)	17.2 (5)	17.5 (4)
21 6–26.2	1_3–25 6	14 0–18.0
7.354 (3)	7.349 (3)	7.296 (4)
7.340–7.365	7.259–7.367	7.200–7.373
32 (4)	28 (3)	30 (4)
29–33	25–35	4–39

Arterial blood

pCO_2 (mmHg)	pO_2 (mmHg)	pH
31	94	–
	–	–
36	109	
34	87	7 46
32	77	
36	102	7 42
	–	
35	–	
35	73	7 475
39	57	–
33	126	7 455
40	81	7 453
41	143	
33	149	
34	93	7.380
6	86	
7	55	

The increased CSF lactate concentration

In patients with bacterial meningitis the increase of clactate(CSF) may be due to the presence of leucocytes and bacteria in the CSF as suggested by Montard and Perret (9) This suggestion was supported by the parallel decrease of the leucocyte and lactate concentrations of the CSF It was further supported by previous *in vitro* studies on lactate production of leucocytes at rest and during *phagocytosis* (3 15) Other factors might contribute since clactate(CSF) was still increased at the time of the third sampling when the CSF leucocyte concentration was only slightly elevated in the majority of cases Slow clearance of lactate from CSF (10) might be one possible explanation. Increased lactic acid production in the brain tissue due to inflammation might be another Furthermore assuming that hyperventilation was caused by some other mechanism than CSF acidosis hyperventilation could by itself lead to increased clactate(CSF) (17)

In severe cases of encephalitis the clactate(CSF) increase could probably be explained in part by lactic acid production in inflammatory brain tissue and partly by the presence of decerebrate rigidity and convulsions the latter of which may be accompanied by arterial hypoxemia (7) Hypoxemia leads to an increased production of lactic acid in the brain. The CSF leucocyte concentrations were in every case of encephalitis so low that the leucocytes could hardly account for the presence of lactate

Mechanism of hyperventilation

It is well documented that the pH(CSF) is a major factor in the regulation of respiration (5 11) The respiratory centers are extremely sensitive to pH changes. In patients with bacterial meningitis 29 pH measurements of lumbar CSF were carried out during spontaneous respiration with pCO_2(aB) below 40 mmHg. Seventeen cases showed a pH(CSF) below 7.30, five had a pH within the normal range (7.30–7.34) and seven had a pH above 7.34 Thus CSF acidosis could explain the hyperventilation in a substantial part of the observations.

However since the hyperventilation in a number of cases remains unexplained other mechanisms must be looked for—first of all the possibility of errors in pH(CSF) due to loss of CO_2 during sampling or measurement. Although care taken to avoid such errors, CO_2 loss

overestimation of the pH(CSF) may have occurred

Because cisternal CSF samples were always taken during short-lasting artificial ventilation in connection with blood flow measurements only lumbar CSF samples obtained during spontaneous respiration were considered in establishing the relationship between pCO_2(aB) and pH(CSF) However Plum and Price (14) recently reported differences between cisternal and lumbar CSF samples with considerable individual variations but generally with lower pH and higher pCO values in the lumbar samples They concluded that lumbar CSF samples may provide unreliable information about cerebral acid–base status Therefore our data cannot settle the question whether hyperventilation may be found in meningitis or encephalitis without local acidosis at the level of the respiratory centers.

Besides a CSF acidosis other factors could possibly stimulate respiration in these patients. Arterial hypoxia increases the ventilation when the pO_2(aB) is reduced to below 60 mmHg. Such low pO_2(aB) values were found in three patients only Hypoxia was therefore an unlikely explanation in the majority of cases

Raised intracranial pressure may be accompanied by hyperventilation Since the lumbar CSF pressures were frequently about 400 mm H_2O in atients with bacterial meningitis this factor might of importance

lly the possibility of a neurogenic nism should be mentioned. In cerebral apoplexy Lane et al (4) found a negative correlation between pCO_2(aB) and pH(CSF) in lumbar spinal fluid samples They considered this finding to provide evidence that a neurogenic mechanism" is responsible for hyperventilation. We did not find a similar correlation in patients with meningitis but five observations in encephalitis fitted a negative correlation ($p<0.02$) The number of observations and the fact that they concerned lumbar CSF samples did not, however allow extensive conclusions

To sum up we were not able to demonstrate acidosis in lumbar CSF in every case of hyperventilation but this does not necessarily exclude local acidosis at the level of the respiratory centers. Increased intracranial pressure was a possible stimulus in meningitis and a neurogenic mechanism could not be ruled out in encephalitis.

THERAPY CONSIDERATIONS

Symptomatic therapy in bacterial meningitis and in encephalitis aims at maintaining ventilation and circulation at a level that offers the best chances of survival for the brain tissue

Respiratory insufficiency may arise gradually from impairment of cough and/or swallowing reflexes in comatose or somnolent patients or from damage of the central mechanisms regulating respiration In both cases manifest respiratory failure must be treated by means of artificial ventilation.

The pCO_2(aB) in patients with meningitis or encephalitis was frequently below 25 mmHg. Most of the patients did well and eventually recovered but in some cases an extremely bad cerebral condition was connected with pCO_2 values somewhat higher although still below the normal value of 40 mmHg. In such cases artificial respiration should be started without hesitation even if the patient does not show the above mentioned types of respiratory failure

It is questionable at what level pCO_2(aB) should be kept during artificial ventilation. A low pCO_2 is probably beneficial in order to counteract CSF acidosis to reduce intracranial pressure and possibly to restore autoregulation (13) On the other hand extremely low pCO_2 levels may cause cerebral ischemia A reasonable and safe compromise is probably a pCO level of 30 mmHg.

The connection between CSF acidosis in meningitis or encephalitis and the regulation of cerebral blood flow has been discussed in another part of the present study (1) It should be mentioned here only that, in addition to a 'relative hypercapnia arterial hypertension and hypoxia appear dangerous factors in these patients.

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EPSTEIN-BARR VIRUS (EBV) INFECTIONS IN ARMY RECRUITS

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Abstract Serum specimens collected from 477 recruits entering military service have been studied for fluorescent IgG antibodies to Epstein-Barr virus (EBV). Only 40 men (8.5%) were negative and 91.5% had antibodies to EBV During the 8–11 months military service repeated serum specimens were taken from 35 antibody negative men and at every infection of the upper respiratory tract. During this follow-up period 4 negative men (11 4%) had seroconversion One of them had typical heterophil antibody-positive infectious mononucleosis, two had had tonsillitis 3 and 4 weeks before the specimens with seroconversion were taken, none of these three men had heterophil antibodies. The fourth man reported no illness. Serum specimens from all four recruits with seroconversion were studied also for fluorescent IgM gel-precipitating and complement-fixing antibodies to EBV These tests confirmed the diagnosis of EBV infection.

Henle et al (3) published in 1969 the first report of EBV as the etiological agent of infectious mononucleosis Patients with heterophil antibody positive infectious mononucleosis regularly developed antibodies to EBV which were clearly distinguished from heterophil antibodies. This disease is recognized as a common disease in older children and young adults especially in college students (1 9). Lehane (7) reported that among marine recruits and Vietnam veterans the rate of EBV seroconversions was 18 5–23 5/1 000 during the military service. Only one tenth were hospitalized for infectious mononucleosis whereas the remaining cases were more or less silent infections.

The present report describes the incidence of both silent EBV infections and the clinical infectious mononucleosis in two groups of army recruits altogether 477 men After the initial serum survey the EBV antibody-negative men were studied serologically and clinically throughout the service period of 8–11 months

SUBJECTS AND METHODS

Two groups of army recruits serving at the Military Training Center in Turku, Finland were chosen for this study The first group entered military service in Oct. 1970 and the second in Feb 1971 at the average age of 20 years. The total number of men studied was 477 A serum specimen was taken from each recruit entering military service, and the specimens were tested for fluorescent IgG antibodies to EBV by the method of Henle and Henle (2) with modifications (6) Altogether 40 of the men (8 5%) were negative and thus considered to be susceptible to EBV infection. During the follow-up period serum specimen was taken from 35 recruits at 3-month intervals during the whole military service, and at every infection of the upper respiratory tract. Five of the 40 men dropped out from the study soon after the first serum sample was taken because of furlough or transfer to another training center the remaining 35 men were followed up throughout the whole military service (8–11 months) When seroconversion was detected among this group the clinical picture was clarified carefully The serum specimens of the recruits with seroconversion were tested also for fluorescent, EBV-specific IgM antibodies by the method of Schmitz and Scherer (16) with modifications (10).

In this test the Burkitt cell line P3HR 1 was used. The cells were kept at 3?°C for 14 days before they were used for slides The acetone-fixed slides were overlaid with serum dilutions, incubated in a moist chamber at 37°C for 3 hours. After washing with phosphate buffered saline (PBS) the slides were dried and overlaid with anti-human IgM conjugate (Wellcome) at 1 20 dilution and incubated again for 45 min. The slides were then washed again with PBS, mounted provided with cover-glass and read off

All sera from recruits with seroconversion were tested for gel-precipitating (GP) and complement fixing (CF) antibodies to EBV too. The antigen for GP and CF tests was made from P3HR 1 cells after treatment with Tween 80 (11 12) The heterophil antibodies were studied by the horse erythrocyte agglutination slide test (5). The positive sera were titrated in twofold dilution by means of slide agglutination.

Table I. *Results of EBV antibody titrations in serum from 4 recruits with seroconversion*
FA = fluorescent antibodies

Pat. no	Weeks after beginning of military service	FA IgG	FA IgM	CF	GP Lines	GP Grade	Horse erythrocyte agglutination
1	0	<10	<10	<5	0	0	–
	8	<10	<10	<5	0	0	–
	23	<10	<10	<5	0	0	–
	32	640	320	10	1	1	+1/8
	34	640	320	10	2	1	+1/4
	42	160	<10	20	2	2	–
	44	160	<10	20	2	2	–
2	0	<10	<10	<5	0	0	–
	9	<10	<10	<5	0	0	–
	26	320	<10	10	1	1	–
	36	160	<10	10	1	1	–
	43	160	<10	10	1	1	–
3	0	<10	<10	<5	0	0	–
	9	80	40	10	1	1	–
	20	80	<10	20	2	2	–
	26	160	<10	20	2	2	–
	40	160	<10	20	2	2	–
	52	160	<10	20	2		–
4	0	<10	<10	<5	0	0	–
	9	40	<10	<5	0	0	–
	10	40	<10	<5	1	1	–
	19	80	<10	<5	1	1	–
	26	40	<10	<5	1	1	–
	36	40	<10	<5	2	2	–
	43	40	<10	<5	2	2	–

RESULTS

In the study population of 477 men 91.5% had fluorescent IgG antibodies to EBV. In the follow- group of 35 men without antibodies 4 (11.4%) oped antibodies to EBV during the military ice one of whom had a typical infectious nonucleosis and 3 a more or less atypical clinical picture.

CASE REPORTS

Case 1

A 19-year-old farmer living in the country was negative for EBV antibodies on April 6th 1971 in the 23rd week of military service. He visited the doctor on June 7th in the 31st week of military service because of a headache, chest pain and slight fever of 38°C and was admitted to hospital. ESR was 3 mm/h. Hb 160 g/l, serum creatinine 116 µmol/l. Urinalysis and chest X-ray were normal. On June 11th, after 32 weeks of service, WBC was 6700, bands 0.02, segs 0.25, lymphs 0.65, monos 0.08 and the horse erythrocyte agglutination was negative. The serum revealed fluorescent IgG and IgM antibodies and both CF and GP antibodies to EBV (Table I). At physical examination he had a slight swelling under the ear and at the ramus of the jaw on the left side and enlarged cervical glands on both sides. Tonsil were red and enlarged with white spots. The left side of the pharynx was swollen and peritonsillar abscess was suspected, but the consulting otologist excluded this and confirmed the diagnosis of infectious mononucleosis. The patient was sent to his unit and bed rest was ordered. On June 14th ESR was 18. The patient recovered rapidly without any complications. The next serum specimen was taken on June 24th, in the 34th week of service, and revealed the same antibody titers to EBV except that one more GP line had appeared and the horse erythrocyte agglutination titer had dropped from 8 to 4. On Aug. 25th, in the 42nd week, the IgG antibody titer had decreased from 640 to 160 and neither IgM antibodies to EBV nor heterophil antibodies were detectable. The CF titer had increased as also had the intensity of GP reaction. In the 44th week, just before the end of military service, his titers remained constant.

Case 2

A 19-year-old garage worker living in town was negative for EBV antibodies on April 19th 1971 after 9 weeks of military service. He was sent to another training center and had been admitted to the local hospital on July 4th for 3 days because of diarrhea, vomiting and abdominal pain. At physical examination the abdomen was diffusely tender on palpation. ESR was 3 mm/h and temperature 37.3°C. He recovered completely after 2 days. The diagnosis of acute gastroenteritis was made. The next specimen taken on Aug.

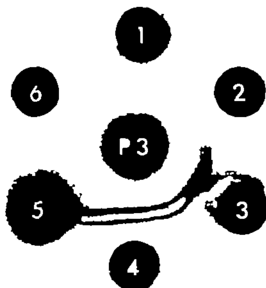


Fig. 1. Development of GP antibodies to EBV in serum from patient 3. *1*=preillness serum, taken at entry to military service. *2*=in 9th week, *3*=in 20th week, *4*=in 26th week, *5*=a control serum with weak activity. *6*=PBS, *P3*=EBV antigen from the Burkitt line P3HR-1.

26th, in the 26th week of service, was positive for IgG, CF and GP antibodies to EBV (Table I). No IgM antibodies to EBV or heterophil antibodies were detectable. It is possible that the EBV infection was subclinical or with very slight symptoms and the gastroenteritis was caused by some other agent.

Case 3

A 20-year-old welder, living in town, was negative for EBV antibodies on admission into the service on Feb. 15th, 1971. The second specimen taken on April 19th, in the 9th week of service, was positive for EBV IgG, IgM, CF and GP antibodies, but not for heterophil antibodies (Table I). On March 15th he had had tonsillitis with 39°C fever for 1 day and was admitted to hospital. ESR was 34 mm/h on the next day and 4 mm/h 3 days later. He was treated with phenoxymethylpenicillin 800 000 U every 6 hours by mouth, and was sent to his unit after 5 days. It is highly probable that this infection had been caused by EBV, because the late convalescent serum taken 4 weeks after the illness still contained IgM antibodies to EBV. The third specimen taken on Aug. 26th, in the 20th week of service, did not contain IgM antibodies, but the CF and GP antibody reactions were stronger than in the earlier specimens (Fig. 1). The specimens of the 40th and 52nd week revealed the same antibody levels.

Case 4

A 19-year-old merchant, living in town, was negative for EBV antibodies at the beginning of military service on Feb. 15th, 1971. The second specimen taken on April 19th, after 9 weeks of service, was positive for IgG antibodies to EBV but negative for heterophil antibodies. No IgM or other types of EBV antibodies were detectable. On March 25th he had had tonsillitis and was admitted to the garrison hospital for 4 days. Temperature was 38–39°C for 3 days and ESR 25 mm/h. He was treated with phenoxymethylpenicillin 800 000 U every 6 hours by mouth. He recovered rapidly and was sent to his unit. In the serum specimen taken on April 28th, in the 10th week, one week later than the previous specimen, there were already GP antibodies. He did not develop CF antibodies to EBV but the GP reactions became still stronger up to the 36th week (Table I).

All these recruits had a clear seroconversion not only for IgG but also for GP antibodies which are sometimes clearly demonstrable (Fig. 1). Only three men had a seroconversion for CF antibodies to EBV. The micro GP technique has also earlier been demonstrated to be more sensitive than the CF technique for EBV antibody detection when the crude antigen preparation is used (11). Both the GP and CF antibodies developed slowly, which has some advantages in this type of follow-up. These antibodies are in general detectable 4–6 weeks after the onset of disease, which makes it possible to determine the time of the acute phase of infection retrospectively. The IgM antibodies to EBV were detectable in two patients (nos. 1 and 3) (Table I). In the two others the IgM antibodies had already disappeared. Only one patient (no. 1), who had the typical infectious mononucleosis, had also heterophil antibodies. The serum specimens from all 477 recruits were titrated for fluorescent IgG antibodies to EBV. Initially 437 men, 91.5% were positive for EBV antibodies. Since most of the recruits are about 20 years old, also the majority of the negative recruits belonged to this age group. The small number of recruits younger or older than 20 did not enable one to calculate statistically the rate of EBV positiveness in the other age groups. The distribution of antibody titers of the whole population of recruits at entry into military service is shown in Fig. 2. The titers most commonly varied between 80 and 320, 77.5% of the titers falling within this range. The immunological profile (13) of the whole material of recruits is shown in Fig. 3 and the median titer 174 is calculated from the figure.

In earlier studies on EBV antibodies it has been reported that the prevalence of antibodies is highest in the low socio-economic groups. In this

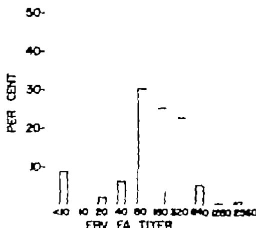


Fig 2 Distribution of fluorescent IgG antibody titers to EBV in serum from 477 army recruits of average age 20 years

study the recruits were divided into three social groups according to Rauhala (15). Because very few of them belonged to group I this and group II have been combined as the higher socio-economic level group III forming the lowest level (14). In this study there was some difference in the percentage of EBV negative recruits in the higher and in the lower social groups. The higher group had more negative men than the lower but the difference was not statistically significant. The material was also classified according to the place …idence but there was no significant differ… between the rural and urban recruits as far … prevalence of antibodies is concerned … the recruits were divided into three groups according to education in the army as men, non-commissioned officers and officers no statistical differences were noted

DISCUSSION

In the present study EBV infections were followed during the military service in two groups of army recruits These infections were less common than expected. Taking into consideration that 4 (11 4%) of 35 men who were susceptible to this infection fell ill within 11 months it was however not especially rare among the susceptibles Only one recruit had a typical infectious mononucleosis with heterophil and IgM antibodies to EBV Two recruits had a tonsillitis shortly after entering military service when results from the antibody titrations of the first serum specimens were not yet available Thus we lack the acute phase specimens from those two The late convalescent serum specimens taken 3–4 weeks after the onset of disease are heterophil antibody-negative One of these two had IgM antibodies to EBV the other was already negative It is highly probable from the clinical data and from the EBV antibody response that these infections were caused by EBV The only infection of the fourth recruit with a seroconversion was a gastroenteritis 7 weeks before the EBV-positive serum specimen was taken. This sample was also negative for heterophil antibodies It is possible that this disease was caused by EBV but it is perhaps more probable that the EBV infection was subclinical

Lehane (7) has reported that the inapparent infections are ten times more frequent than the apparent All of our four patients with seroconversion developed GP antibodies but only three had CF antibodies to EBV These two methods are convenient in this type of follow-up, because the GP and CF antibodies develop slowly The GP and CF antibodies are detectable usually 4-6 weeks after the onset of disease and keep rising up to about 16 weeks in some cases as reported earlier (11). By these methods combined with the disappearance of IgM antibodies to EBV it is possible to determine the approximate time of the acute phase of the disease Thus the symptoms of clinical infections can be studied retrospective

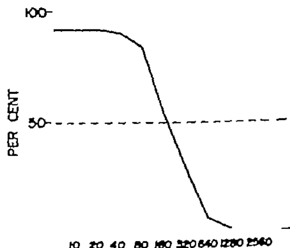


Fig 3 Immunological profile of fluorescent IgG antibody titers to EBV in serum specimens from 477 army recruits. The crossing of the 50% level and the immunological profile indicates the median titer 174.

ly. In the case of an EBV infection this might be valuable because the symptoms are variable from subclinical infections to clinical infectious mononucleosis.

EBV infections do not seem to be especially common among Finnish army recruits as a whole since about 93% are already infected at that age. As high a prevalence as this is in the comparable age group has been reported from Sweden (17), from the USA (Chicago and Alaska) (18) and from Japan (4). In Lehane's study (7) the prevalence of EBV antibodies was 86% and the rate of seroconversions during military service was about three times higher than in our material: 4/477 (0.84%). However, Niederman et al. (8) have reported that the total incidence of EBV infections per year among suspectible college students is 11.1%, the same as in the present study: 11.4% (4/35). They also reported that the inapparent-to-apparent case ratio was 1:2 as compared to 1:3 in this sutdy. There are reports in which people with low socio-economic background have had antibodies to EBV more often than those from high social groups (3, 9). There was a similar tendency in the present findings but the difference was not significant.

ACKNOWLEDGEMENT

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CASE REPORT

The patient is a woman born in 1918 Her father died at the age of 81 in a leukemic disease and brother at the age of 45 of renal carcinoma. She underwent two operations on the spinal column in 1946 and 1949 because of scoliosis and herniated lumbar disc respectively Polyps in the rectum were locally resected in 1954 and 1955 I 1968 she was unsuccessfully subjected to dietary treatment of obesity during 4-week period as in-patient Because of an urtis plasma protein analysis was performed in 1969 The analysis was done by the Clinical Laboratory of the University Hospital of Uppsala and revealed clearly subnormal levels of IgG and IgM with IgA just below the normal range. The following values were reported. IgG 4.7 IgA 0.8 and IgM 0_ g/l. No M component was found. Unfortunately no test for Bence Jones proteinuria was performed.

In 1970 a carcinoma of the transverse colon was detected and colectomy was successfully performed No evidence of metastases was found at the operation and the ileostomy has been functioning well since then. Her weight has remained constant.

I Jan 1974 she came a out-patient to the department of Internal Medicine of Avesta Hospital complaining of moderate pain in the back and the legs. The physical examination revealed little except the previously noted obesity BP wa 160/90 Laboratory investigations yielded the following results: ESR 3 mm/1 hour Hb 14 4 g/100 ml, erythrocytes 4 400 000/mm³ leucocytes 5300/mm³ eith normal differential, platelets 178 000/mm Plasma protein analysis was performed by the Clinical Laboratory of the University Hospital of Malmö and again revealed the same pattern of immunoglobulin deficiency as in 1969 The following values reported IgG 4 IGA 1 0 and IgM < 0.2 g/l No component or any other protein abnormality wa Urine electrophoresis performed by the same y revealed 175 mg/l of Bence Jones protein of kappa type

Because of the finding of light chains in the urine the patient was admitted to the hospital as in-patient with the presumptive diagnosis of light chain disease A complete roentgenological examination of the axial skeleton as well as of the long bones of the extremities was performed without any unequivocal evidence of any destructions. Because of slight irregularity of the right seventh rib, an extra X-ray was taken of this area. N destruction was found however Serum creatinine was 1.09 mg/100 ml, serum calcium 5 0 mEq/L Liver function tests were all normal Bone marrow from the sternum was sent to I Granberg at Huddinge Hospital who reported completely normal findings without any increase in the number of plasma cells The patient was discharged with the presumptive diagnosis of idiopathic, benign light chain disease

DISCUSSION

Immunoglobulin deficiency without an M component in the plasma combined with Bence Jones proteinuria is characteristic of the majority of cases of light chain disease (4) In our patient the pattern of immunoglobulin deficiency has remained more or less constant over a 5-year period Bence Jones proteinuria has been detected at the beginning of 1974 No test for light chains in the urine had been performed previously We have found no other clinical evidence of myelomatosis in this patient She continues to be in a non-deteriorating clinical condition with slight symptoms attributable to her overweight.

This patient may of course after an initial period of many years of a steady level of immunoglobulin abnormalities later develop progressive light chain proteinuria together with the clinical picture of myelomatosis She may still be in a dormant, premyelomatous stage of an eventually malignant plasmacellular dyscrasia.

Another possibility is that she may have a solitary plasmacytoma that cannot be clinically localized Hobbs (1) has reported that the hypogammaglobulinemia of a patient with a solitary plasmacytoma of the head of the femur rose to normal levels after the tumor had been removed and remained normal two years later He is of the opinion that a solitary plasmacytoma may release a humoral factor which can depress the protein production of the normal plasma cells. Theoretically therefore even in solitary plasmacytomas not detectable by ordinary clinical methods, signs of disturbed immunoglobulin metabolism could be evident. In our case we would have to postulate a light-chain-producing, solitary plasmacytoma.

A third and equally intriguing possibility of course, is that she may have a truly idiopathic light chain dyscrasia. Only continued follow-up may give us the chance to determine whether one of these three alternative hypotheses will prove to be correct or whether another type of disease associated with Bence Jones proteinuria will ultimately develop.

In our present opinion however the fact that the plasma protein abnormalities of our patient have remained more or less constant over a 5-year period speaks in favour of a benign plasmacellular dyscrasia. In a case of light chain disease it would be exceedingly unusual not to have definite progression of abnormalities within such a long period We should therefore like to suggest that our patient has a form of benign light chain dyscrasia, a hitherto unrecognized type of benign gammapathy

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Announcements

An International postgraduate course on clinical cardiology will be held in Davos Switzerland, Feb 16–21 1975 Organized by Dr B Pitt, Associate Professor of Medicine The Johns Hopkins Hospital Baltimore Maryland USA in cooperation with lecturers from the USA and Europe. Sponsored by the American Heart Association

Subject. Diagnosis and therapy of angina pectoris and myocardial infarction.

Further information and registration forms (European participants): Prof Dr P Lichtlen Abteilung für Kardiologie Department für Innere Medizin Medizinische Hochschule Hannover D-3000 Hannover BRD (Participants from overseas) Dr B Pitt, Associate Professor of Medicine Division of Cardiology The Johns Hopkins Hospital Baltimore Md USA Number of participants is limited Closing date for registration Dec 31 1974

International Symposium on Intensive Therapy total parenteral alimentation, will be held in Rome Italy May 30–June 2, 1975

Organizing secretariat International Symposium on Intensive Therapy New Media Via Giovanni Battista Martini 6 I-00198 Rome Italy

The Sixth International Congress of Nephrology will be held at the Palazzo dei Congressi in Florence Italy June 8–12, 1975

Scientific Program Committee Nephrology Dialysis Dept. S Orsola University Hospital Via Massarenti 9 I-40138 Bologna, Italy

Social Program Committee Institute of Urology University of Florence Viale Pieraccini 18 I 50139 Firenze Italy

THANKS TO OUR REFEREES

During the last two years when I have been Chief Editor of this journal, the referee system has been used systematically The Editors want to express their gratitude towards the many colleagues who have given time and thought to this task. We hope that we shall have the collaboration of these and other specialists within different fields also for future volumes of our journal Through their efforts many faults have been discovered and many obscure points clarified

It is our hope that the referees will continue to help us to raise the standard of the Acta and we believe that this journal—and thus its readers—have profited greatly from the suggestions and positive criticism.

Anonymity has been condition for unbiased work as a referee. A list of their names gives us a chance to remember their fforts without unmasking the ndividual

P Bechgaard, K. Brochner Mortensen, V Faber J Hess Thaysen K. Iversen Sv Aa.Killmann V Posborg Petersen, J Poulsen, M Schwartz, A Tybjerg Hansen N Tygstrup P Halonen, W Karpainen, B -A. Lamberg, E. A Nikkilä, O Wegelius, E. M Blegen, O. J Broch E. Enger O Storstein, S Almqvist U T Axelsson S Å Belfrage U Bengtsson S E. Bergentz, J Bergström, Å. Bertler G Biörck, P Björntorp L.-O Brandt, L. A Carlson J Dymling, S Efendić, H. Elfasch L. Engstedt, L. R. Erhardt, S Eriksson H. Forssman L. Garby S Gardell, G Gahrton A. Gustafsson K Gydell K Hall, L. Å Hansson, R. Hed, S Hofvendahl, C Högman B Hökfelt D Ingvar B Johansson, A Killander C B Laurell, T Leonhardt S Lindstedt J E. Lindsten J G Ljunggren T Lundberg, B Lundh, T Lundman, R. Luft, G Magnusson H Malmros V Mutt, H Möller B Nilsson I M Nilsson, B Nosslin E. Orinius, B Pernow H Reichard P Reizenstein A Rosén, B Scherstén H Sjöberg, A Sjögren F Sjöqvist N Söderström N Törnblom L. Werkö P O Wester L. W Wilhelmsen.

Acta Medica Scandinavica thanks its many competent referees and wishes them as well as our authors and readers a happy New Year 1975

J n G Waldenström

Acta med scand Vol 196 pp. 451–458 1974

ACETYLSALICYLIC ACID INTOLERANCE

Lack of N-acetylsalicylic Acid Specific Skin-sensitizing Antibodies in the Serum of Intolerant Individuals

H D Schlumberger E A. Löbbecke and P Kallós

From the Institute for Imm nology and Oncology Bayer AG Wuppertal, West Germany and Helsingborg S eden

Abstract Twenty-seven ASA-intolerant individuals were investigated. Twenty-six of them suffered from chronic bronchial asthma, one from urticaria. All reacted to the ingestion of therapeutic dose of ASA with more or less violent signs and symptoms resembling a systemic allergic (anaphylactic) reaction of the immediate type Seventy percent of them reacted in the same way to one or more chemically unrelated drugs. Seventeen of these patients were skin-tested with N-salicyloyl- and N-acetylsalicyloyl-PLL without the appearance of immediate type reactions. Furthermore, the sera of these patients were passively transferred to rhesus monkeys. The intradermally sensitized monkeys were challenged by or intradermal injection of N-salicyloyl-BSA or N-acetylsalicyloyl-PLL or by oral administration of ASAN or ASA. No positive reaction was obtained Intracutaneous sensitization of rhesus monkeys with the sera of individuals allergic to Timothy grass pollen revealed strong reactions of the immediate type following challenge with Timothy grass pollen extract The IgE levels and the concentrations of the other immunoglobulins of the ASA intolerant individuals were found to be withi the normal range. Thus, homocytotropic antibodies with N-salicyloyl or N-acetylsalicyloyl specificity could not be detected in the ASA-intolerant individuals. These results, the frequency of intolerance to other drugs the familial occur rence of ASA intolerance and the demonstration of "latent" ASA intolerance in about 20% of chronic asthmatics indicate that the clinical picture of the syndrome is not sufficient criterion of its allergic origin In our opinion the syndrome is caused by direct (I not antibody mediated) liberation of histamine and other mediators by the offending drugs due to an "inborn error of metabolism in intolerant individuals. The syndrome should be properly denoted as drug-induced anaphylactoid syndrome

Adverse reactions to acetylsalicylic acid (ASA) re sulting in omiting severe asthma attacks angioedema, rticaria, fall of the BP and in few cases lethal shock (13 18 19 28 33 53 54) have been known for more than 60 years (23 45) The major symptoms of these untoward reactions to ASA resemble those of an allergic disorder of the immediate type Therefore it has been assumed that individuals showing such reactions after intake of therapeutic doses of ASA are allergic to it This assumption became understandable by the detection of a potentially immunogenic impurity present in low amounts in commercially available ASA preparations namely acetylsalicylic acid anhydride (ASAN) (12).

Skin testing of ASA-intolerant individuals usually fails to give positive results (24 40 56 59) This and the fact that a great number of ASA-intolerant individuals show intolerance to other chemically unrelated drugs too suggest the involvement of non-immunological rather than an allergic pathogenesis of the majority of adverse reactions to ASA (46). However some cases with adverse reactions to ASA showing positive skin tests have been reported (12, 36 46). Girard et al (25) described 4 cases with a serum sickness type of reaction to ASA two of whom showed an Arthus-type skin test. Lazary et al (36) advanced the hypothesis that urticaria but not the other adverse reactions to ASA are of allergic origin.

In this paper evidence will be presented that in ASA-intolerant individuals no homocytotropic antibodies (3) are detectable either by skin testing with N-acetylsalicyloyl-poly L-lysine (PLL) (12) or by passive transfer of their serum nto rhesus monkeys (34 35)

MATERIAL AND METHODS

Antigens

Antigens used for skin testing of ASA-intolerant individuals and for that of passively sensitized rhesus monkeys

Table I. *Clinical data of ASA-intolerant patients and symptoms after ingestion of ASA*

A=asthma, R=allergic rhinitis, U=urticaria, AE=angioedema, Ec=eczema, Mi=migraine, P=nasal polyps, N=nausea, V=vomiting, UC=unconsciousness, s=severe, ss=very severe, n.d.=not done

Pat. no.	Age (y)	Sex	Original complaint	Reaction after ingestion of ASA	Skin test[a]	Intolerances or allergies against other drugs
1	29	♀		U	Neg.	–
2	45	♀	U[s], Mi	AE, V	Neg.	–
3	76	♀	Ec, Mi	U, R, Vs	Neg.	Sansert®: N, UC
4	59	♀	A, R	As, V	n.d.	Quinidine: V, A
5	57	♂	A	As, V	n.d.	–
6	44	♂	A, R	As, V	n.d.	Indomethacin: V, A
7	63	♀	A, R, P	A, V	n.d.	Indomethacin, Hygroton®: V, A
8	50	♀	A, R, Ec	A, Vs	n.d.	–
9	46	♀	A, R	A, AE, Vs, U	Neg.	Barbiturates: V, U
10	67	♀	A	AE	Neg.	–
11	51	♂	A, R, P	A, AE, V	n.d.	Sulphonamides: A, AE, V
12	53	♀	A, U	A, U, Vs	Neg.	Penicillin: A, U, Vs
13	47	♀	A, R, Mi	A, V	n.d.	Penicillin: U
14	46	♀	A, R	A, V	n.d.	Penicillin: A
15	80	♀	A	Ass, V	Neg.	Penicillin: UC, low BP
16	52	♀	A, R	As, V	n.d.	Saridon®: As, V
17	55	♀	A, R	As, V	Neg.	Sulphonamides, quinine: A, V, vertigo
18	54	♀	A	A, Vs, vertigo	Neg.	Indomethacin: A, V, vertigo
19	48	♀	A, R	As, Vs	Neg.	Sulphonamides: A, Vs; penicillin: U
20	59	♀	A, AE, Mi	As, AE, V	Neg.	Indomethacin, Recipavrin®, penicillin, erythromycin, iodine, quinine, Lasix®, quinidine (also peas, apples, onions, grape fruit): A, AE, Vs
21	60	♀	A, R, U, AE	A, R, U, AE, V	n.d.	Hygroton, Salures, Isotonyl®, quinine, sulphonamides: A, R, U, AE, V
22	65	♀	A	Fixed xanthema	Neg.	–
23	53	♀	A, R	A, AE, V	Neg.	–
24	25	♂	A, U, Ec	Ass, N, V	Neg.	–
25	50	♀	A	A, AE, V, shock	n.d.	Novocain®, Xylocain®: shock
26	50	♂	A	A, AE, V	Neg.	Saridon®: V, AE
27	79	♀	Hay fever	AE, UC	n.d.	Novocain, Xylocain®: UC

[a] With N-acetylsalicyloyl/salicyloyl-PLL. [b] Thermal.

conjugates of ASA or salicylic acid with PLL (n=40) or with bovine serum albumin (BSA): 1) N-acetylsalicyloyl/salicyloyl-PLL in which 25% of Σ-amino groups of the PLL were reacted with N-acetylsalicyloyl-hydroxysuccinimide ester. The residual Σ amino groups were succinylated. 2) N-acetylsalicyloyl/salicyloyl-PLL in which 50% of the Σ-amino groups were salicyloylated by reaction of PLL with ASA chloride. 3) BSA was reacted with salicylic acid nitrophenyl ester resulting in a conjugate which contained 13–14 N-salicyloyl residues/mole BSA. The antigen solutions were sterilized by filtration and lyophilized in sterile ampoules.

The antigens used in skin testing of ASA-intolerant individuals and in the passive transfer studies have been prepared and chemically characterized by Dr E. Schnabel, Biochemical Laboratory, Bayer AG, Wuppertal (48). The antigens employed were assayed for their anaphylactogenic activity in guinea pigs which were sensitized intradermally with ASAN dissolved in dimethylsulphoxide according to De Weck (12). All animals sensitized on days 1, 3, 6, 9 and 13 with 1 mg ASAN exhibited systemic anaphylactic reactions after i.v. challenge with the N-acetylsalicyloyl or N-salicyloyl conjugates (antigens 1, 2 and 3).

Skin testing of ASA-intolerant individuals

Skin tests were carried out by intradermal injection of 0.02 ml of a 1% solution of antigens 1 and 2.

Passive transfer of sera of ASA-intolerant individuals to rhesus monkeys

Rhesus monkeys (Macaca mulatta) weighing 2.5–3 kg were used for passive transfer experiments. To visualize the antigen-antibody reaction in the passively sensitized skin after antigen challenge the animals received 1.1–1.5 ml/kg of a 0.5% Evans blue solution in buffered saline either simultaneously with the antigen or 15–20 min after intradermal or oral application of antigens or haptens according to Layton et al. (34, 35). The sera tested in these transfer experiments were filtrated through 0.22 μ Millipore filters and stored in portions of 0.5–1 ml in sterile ampoules at −20°C. Trials for the presence of skin-sensitizing antibodies in the sera of ASA-intolerant individuals were performed according to the following experimental records.

Table II *Synopsis of clinical data of ASA-intolerant patients*

Reference no.	44		17		20		56		43		27		46		4		45		16		[illegible]	
		%		%		%	n	%		%		%		%		%		%		%		%
Males	22	35	17	34	17	38	26	31	1	43	32	40	78	43	11	48	20	43	1	40	1	33
Females	40	65	33	66	28	6[illegible]	57	69	16	57	49	60	104	57	12	52	26	57	18	60	4	67
Total	62		50		45		83		28		81		182		23		46		30		36	
Age ≥30 y	41[a]	66	–	–	32[a]	71	46[c]	55	23	82	69[d]	85	136	75	13	57	34[e]	74			14[f]	39
Chronic asthma	43	69	46	92	45	100	78	94	28	100	81	100			23	100			24	80		
Nasal polyps	21	34	33	66	21	47	33	40	9	32	47	58	97	51	11	48	15	33	30	100		
Reactions after ingestion of ASA																						
Asthma	38	61	33	63	45	100	73	88	24	86	80	99	154	85	20[b]	87	15	33	12	40	2	6
Asthma and urticaria and/or angioedema	5[b]	8				–	1	1	–	–	1	1	10	5	3[b]	13			5	17	14	39
Angioedema and/or urticaria	13	1	13	25	–	–	10	11	4	14			18	10			20	43			20	55
Others	6	10	6	1	–	–	4	5	–	–		–					11	4	13	43		
Intolerances or allergies to other drugs	19	31	18	36	13	29	13	15	17	61		–	42[b]	23	2	9	14	30	1	70	1	58

Range 30–50 years. Including asthma + vasomotor rhinitis and asthma + abdominal cramps. ≥40 years. ≥35 years. Including seasonal and/or environmental inhalants. Wheezing, dyspnoea. ≥70 ye[illegible]. Range 30–40 years.

Experiment I. 0.05 ml of each of 17 sera of ASA-intolerant patients were injected intradermally and the injection sites marked. After an interval of 24 hours 10 mg of N-salicyloyl-BSA or 10 mg of N-acetylsalicyloyl-PLL were applied i. v. together with Evans blue. As control, 0.05 ml of normal human serum was applied intradermally. Immediately prior to the injection of Evans blue, histamine dihydrochloride was given intradermally in concentrations of 10, 20 and 30 μg/ml calculated as histamine base. The animals were kept under observation for 6 hours after antigen challenge.

Experiment II. Passive transfer of patient serum was carried out as in experiment I. Antigen challenge was performed 24 hours later by intradermal injection of 0.05 ml of 1% solutions of N-salicyloyl-BSA or N-acetylsalicyloyl-PLL into the marked sensitized skin areas. Normal human serum was used as the negative control. The antigens used for challenge did not induce reactions when injected into non-sensitized areas of the skin. Histamine was given in the same concentrations as described. Evans blue was applied 15–20 min after intradermal antigen challenge. The animals were observed for 6 hours.

Experiment III. The experimental procedure and the controls were the same as in experiment I and II. Antigen challenge was performed 24 hours after skin sensitization by oral application of 50 mg ASAN to one animal and 500 mg ASA to another. Evans blue was injected 15–20 min after ingestion of ASAN or ASA. The animals were observed for 7–8 hours.

To demonstrate the skin-sensitizing properties of human homocytotropic antibodies in primates, 0.05 ml of the serum of two individuals sensitive to Timothy grass pollen were injected into the skin of rhesus monkeys. The serum was applied in original concentration and diluted 1 : 10 and 1 : 100 with buffered saline. 0.4 ml Timothy pollen extract were given i. v. together with 0.5% Evans blue (1.5 ml/kg) 24 hours after sensitization of the skin. Normal human serum and histamine were applied as described. The serum of Timothy pollen sensitive patients and the Timothy pollen extract (V tman 81153) were gifts from Prof. Dr G. Johansson, Uppsala.

Determination of immunoglobulins

The concentrations of IgG, IgA, IgM and IgD in the sera of 19 ASA-intolerant patients and 20 healthy human individuals were estimated by the radial immunodiffusion technique using Immunoplates® (Hyland-Travenol GmbH, München). IgE was determined by radioimmunosorbent test (Phadebas® test, Pharmacia, Uppsala). Employing the standard according to the British Research Standard for Human Serum Immunoglobulin E, 68/341, the IgE concentrations were calculated on the basis of 2 ng/U.

RESULTS

The present study is based on observations for several years in 77 cases of ASA-intolerant individuals (22 females, 5 males) who received medical treatment of the diseases listed in Table I. Of the 27

Table III *Personal and clinical data of ASA intolerant individuals and their reactions after ingestion of ASA*

		%
Original complaints		
Asthma (with or without rhinitis)	19	70 4
Asthma, urticaria and/or angioedema	4	14.8
Others	4	14.8
Nasal polyps	2	7 4
Intolerances or allergies to other drugs	19	70 4
Reactions after ingestion of ASA		
Asthma (with or without vomiting)	13	48 2
Asthma, urticaria and/or angioedema	8	29.6
Angioedema and/or urticaria	5	18.5
Others	1	3 7
Vomiting	23	85.2
Reactions with symptoms of the original complaints	21	77.8
Reaction of asthmatics with asthma	21	91.3
Reactions with other symptoms than original complaints	6	22
Reaction of asthmatic with other symptoms than asthma	2	8 7

patients 23 were above 30 years of age Intolerance reactions to ASA and a variety of other drugs structurally unrelated to ASA were explored by thorough investigation of the personal history of these patients and direct observation of the signs and symptoms occurring after intake of ASA or other rugs listed in ble I No provocation tests were ormed becu e these are dangerous

The majority of ur ASA-intolerant patients are emale (81 5%) an include predominantly middle-aged and elderly individuals (85.2%) About 85% of the patients suffered from chronic asthma bronchiale In contrast to a variety of reports in the relevant literature (Table II) only 7 4% of our patients exhibited nasal polyposis In about 85% of our patients vomiting was one of the prominent symptoms of the intolerance reaction

Most of the intolerant individuals reacted to ingestion of ASA with symptoms of their basic disease (77.8%) (Table III). Practically all patients with chronic asthma, except two reacted to ASA with an asthma attack. One patient with asthma exhibited angioedema, another showed a fixed exanthema on the right forearm after taking ASA Patient 1 was seeking medical treatment for her little daughter who was suffering from asthma. Her intolerance to ASA was disclosed during investigation of the family history She had no complaints other than this untoward reaction to ASA to which she reacted with generalized urticaria

Seventeen of our 27 patients intolerant to ASA were skin tested with N-salicyloyl and N-acetylsalicyloyl PLL (antigens 1 and 2) No case showed a positive skin reaction of the immediate type Thus it can be assumed that these patients lack skin-sensitizing homocytotropic antibodies (IgE) with N salicyloyl or N-acetylsalicyloyl specificity The presence of such antibodies were a prerequisite if the syndrome which these patients exhibited after intake of ASA were of allergic origin To confirm these results sera of 17 patients were passively transferred to rhesus monkeys by intradermal injection of 0 05 ml of each serum. The antigen challenge

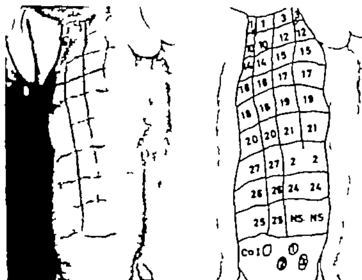


Fig 1 Passive transfer of sera of ASA intolerant individuals into a rhesus monkey The antigen challenge wa carried out by intradermal injection of N-acetylsalicyloyl-PLL into the previously sensitized skin The figures represent the patients sera used *NS*=normal human serum *Co I*=antigen control *1-3* (bottom)= histamine control (10 20 and 30 µg/ The black spots within the marke d areas are additional marks at serum injection

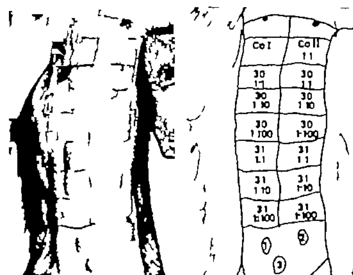


Fig 2 Passive transfer of two sera from patients allergic t Timothy grass pollen (nos 30 and 31) The antigen challenge was carried out by injection of Timoth grass pollen extract simultaneously with Evans blue *C II*=normal human serum Other symbols as n Fig. 1

was carried out either by i v (experiment I) or local (experiment II) application of two different antigen preparations (N-salicyloyl-BSA N-acetylsalicyloyl PLL) or by oral challenge with 50 mg ASAN or 500 mg ASA (experiment III)

The salicyloyl-specific conjugates were tested for their anaphylactogenic activity *in guinea pigs* sensitized with ASAN prior to their use in the monkey experiments. Intradermal application of histamine was used as a control in these experi ments None of the sera tested induced skin reac tions after antigen challenge thus revealing the absence of homocytotropic antibodies specific for N-acetylsalicyloyl or N-salicyloyl determinants Fig. 1 stands as an example for these experiments and *shows the sensitized skin f monk y 30 min* after antigen challenge with N salicyloyl-BSA applied I v together with Evans blu The skin-sensitizing properties of human homocytotropi antibodies in primates were demonstrated by application of the sera of patients allergic to Timothy grass pollens The kin reaction ind ced by I injections of Timothy grass pollen extract together with Evans blue 24 hours after intradermal sens t z tion with the sera is shown in Fig.

The concentrations of the different immunogl bu lins of ASA-intolerant individuals were found t be within the normal range (Table IV) Th applies al to the concentration of IgE which however show *somewhat elevated levels the ASA-i tolera t* group compared with normal individuals In contra t to this the IgE concentrations in the sera of 3 patients with an immediate type allergy to Timothy grass pollens has been found to be significa tly elevated and ranged between 5 600 and 11 000 ng/ml

The presence of immunogenic ASAN in commer cial ASA preparations explains the occurrence f circulating antibodies with N-acetylsalicyloyl *specificity I the serum of ASA-intolerant individu*als and controls previously treated with ASA (1 1 24 57) These antibodies belong to the IgG class and are heterocytotropic (3) I abl to sensitize guinea pigs passively (12, 57). We agree with the conclusion of Amos et al (1) according to which "these antibodies are not involved n an hypersensitivity mechanism

Table III *Immunoglobulin once tration I era of 19 ASA-i tolerant and normal individual (mea ± S.D)*

	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	IgD (mg/100 ml)	IgE (ng/ml)
Normal range	898–1 613 (26)	152 342 (26)	40–220 (26)	0 3–30 (49)	60–1 000 (29)
Normal individuals	1 039±214	114±46	233±66	0 11±0..	186±370
ASA-intolerant patients	1 166±281	197±82	146±55	0 33±1	344±904

DISCUSSION

The majority of patients exhibiting adverse reactions to ingestion of ASA described in the literature suffer from chronic asthma (16 17 20 24 27 39 43 44 56) This is in agreement with our findings so is the fact that ASA intolerance occurs more frequently in females and appears to be manifested preferentially in middle aged patients However Falliers (15) has recently reported 25 cases of ASA intolerance in asthmatic children chiefly in children with late onset of asthma (age range 9–15 y) and high incidence of nasal polyps. In contrast to many reports (Table II) only about 7% of our patients exhibited nasal polyps According to the experience of one of us (P K) patients with chronic asthma frequently exhibit nasal polyps and the incidence of nasal polyposis seems not to be more frequent in ASA intolerant patients

The majority of the ASA-intolerant asthmatic patients react to ingestion of ASA with an asthma attack which is sometimes combined with urticaria and/or angoedema (Tables I and III) One of our asthmatic patients reacted with a fixed exanthema to ASA and another with angioedema only Vomiting occurred in 85% of our cases after intake of the offending drug. Although vomiting has been reported only rarely (28 45) and Pearson (43) denies explicitly that abdominal symptoms pain or vomiting are due to an intolerance reaction it appears to us hat this symptom is a part of the intolerance reaction seems not to be related to the basic disease or to l irritation of the stomach by the drug itself

one of our patients tested with N-salicyloyl-PLL and N-acetylsalicylovl/salicyloyl PLL exhibited immediate type sk n reactions, neither did the passive transfer of sera of ASA-intolerant patients to rhesus monkeys result in positive skin reactions subsequent t i v and intradermal challenge with N-salicyk l-BSA and N-salicyloyl PLL or by oral challeng w ith ASAN and ASA thus indicating the absence of reaginic antibodies in the skin and in the serum of the ASA-intolerant patients. The skin-sens tizing properties of human reaginic antibodies in monkeys are well established by others (34 35) and have been demonstrated by us in control experiments with sera of patients allergic to Timothy grass pollen

Thus our results show the absence of homocytotropic antibodies with N-salicyloyl or N-acetylsalicyloyl specificity in the skin and serum of individuals intolerant to ASA Consequently we must consider that the signs and symptoms occurring after intake of ASA (and in many cases after chemically unrelated drugs) are not of allergic origin but are due to another pathogenetic mechanism Kallós (31) ascribed this syndrome already in 1956 to direct not antibody mediated histamine release through the offending drug due to some inborn biochemical abnormality of intolerant individuals.

The biochemical mechanisms of the drug-mediated intolerance reactions are not known at this time but a variety of hypotheses have been presented Samter and Beers (46 47) discuss a paradoxical reaction of kinin chemoreceptors to ASA and other analgesics resulting in stimulation instead of inhibition of the receptors causing bronchoconstriction and vasodilatation. Generation of anaphylatoxins by direct activation of complement components acting subsequently on release mechanisms of histamine or hypothetical deficiencies of enzyme inhibitors and activation of tissue enzymes by ASA, leading to an imbalance of enzyme and enzyme inhibitors in tissues followed by tissue damage and release of pharmacologically active mediators has been suggested (59). Interactions of analgesic drugs and prostaglandins are also to be considered in the pathogenesis of ASA-induced asthma (42) Prostaglandins of E series have been proved to be bronchodilatatory those of F series are usually bronchoconstrictive (8 50) It has been shown too that ASA and other analgesics inhibit the synthesis of prostaglandins E and F in guinea pig lung (55).

Caplin and Haynes (7) described an abnormal factor in the serum and saliva of ASA-intolerant patients which disorganizes and inhibits the rhythmic beat of gill cilia of freshwater mussels and oysters in vitro within 10–30 min. This factor is regularly present in patients with cystic fibrosis of the pancreas and in less active form in their heterozygous parents The authors discuss on this basis a possible genetic link between ASA intolerance and cystic fibrosis of the pancreas The significance of these results is however doubtful Wood and Di Sant Agnese (58) have recently conducted an objective and thorough evaluation of the reliability of the assay of the circulating cilio-toxic factor in patients with cystic fibrosis of the pancreas and normal controls. They conclude that the "bioassays are highly subjective and completely unreliable in their present form" Obviously these assays have as yet no place among the accepted screening methods for cystic fibrosis (6).

Thus the discussion about the possible biochemical mechanism of ASA intolerance remains open Further investigations are necessary

In the last two years several reports on ASA intolerance in siblings have been published (7 37 41 52). This suggests that genetic factors might be the ultimate cause of the intolerance Strong evidence supporting this view has been provided recently In an ethnic isolate (Mennonites) Lockey et al (37) found a family with high consanguinity Four members of this family (3 first cousins) had asthma nasal polyps and pronounced ASA intolerance The authors stress that the presence of ASA intolerance "in these constellations of relatives suggests that it is inherited as an autosomal recessive trait Were this the case homozygotes would exhibit pronounced intolerance whilst heterozygotes e.g the parents of intolerant individuals, would be free from obvious signs of intolerance and their state could eventually be disclosed by subtle methods such as challenge with ASA under controlled conditions The usually late onset of ASA intolerance suggests the possibility that external factors play a role in its manifestation

McDonald et al. (39) used oral challenge with ASA (0.640 g) in 42 selected patients with chronic bronchial asthma and nasal polyps who were not aware of any drug intolerance especially intolerance to ASA. The challenge has been performed when physical examination and spirometry confirmed the absence of asthmatic symptoms The patients received their usual maintenance medications including cortisone, which usually prevented the occurrence of manifest asthmatic symptoms. After challenge they have been closely observed and spirometric values have been recorded at regular intervals A rapid reduction of 50% o more of the FEV ... indicated a positive response In this w y ASA intolerance could be proved in 8 of the patients They did not react to placebo tablets other analgesics or tartrazine With regard to the findings of Lockey et al (37) these individuals are probably heterozygotes with partial phenotypic expression of their hereditary trait. Another example of this has been provided by Delaney (11) who described a family n which 15-year-old girl presented with severe asthma, nasal polyps and ASA ntolerance. Her father had chronic asthma but denied any drug intolerance Oral challenge with ASA however elicited an asthmatic ttack It is tempting t assume that the father is heterozygote his daughter homozygote Further investigations along these lines are necessary

As mentioned above about 70% of our ASA intolerant cases reacted with identical symptoms to a variety of structurally unrelated compounds too This is in accordance with the observations of several other investigators (16 17 20 22 25 29 43 44 46 51 56) Samter and Beers (46) for instance reported 18 cases of ASA-intolerant asthmatics who reacted w th asthmatic symptoms to therapeutic doses of indomethacin. Previous contact with the drug could be excluded Juhlin et al (30) performed oral challenge in 7 ASA-intolerant individuals with ASA and several other drugs and food additives The patients were aware of their ASA intolerance only A food additive tartrazine (FD & C yellow No 6) in small doses (1–5 mg) elicited se ere reactions (urticaria, angioedema and/or asthma) in all 7 cases Derivatives of benzoic acid elicited similar symptoms in some cases It is perhaps worthwhile to note that ASA free from its anhydride elicited severe reactions in all three cases challenged w th it (doses 3.5–50 mg) It must be assumed that the pathomechanism of the reactions of ASA-intolerant individuals to structurally unrelated compounds is identical w th that operative after ngestion of ASA A number of cases react to antibiotics mainly penicillin too These reactions are probably allergic The signs and symptoms of drug intolerance identical with those occurring anaphylaxis e a more or less violent allergic reaction of the immediate type cannot be regarded as ufficient criterion to implicate an allergic origin A our results and the literature suggest, the syndrome is the sequela of direct liberation of histamine (and other mediators) by ASA and othe drugs. It should be properly denoted as drug-induced anaphylactoid yndrome

The ability of variety f drugs to release histamine in certain ndividual thus nducing the anaphylactoid yndrome is in our opinion due to a hereditary trait During the past few years experimental and clinical studies ha e revealed correlation between hi tocompatibility antigens (HL A and LA in human) and the susceptibility to certain diseases An altered frequency of the occurrence of one or more of these antigens in group of ind viduals suffering from a disease trongly pports the possibility that the susceptibility is due to genetic factors in the host (2 4 5 8 10 14 1 38)

We have started investigations in an attempt to determine whether in the subpopulation of asthmatics showing anaphylactoid reactions after ingestion of ASA and certain other drugs such an alteration of the frequency of HL A or LA antigens can be revealed Recently La Du Jr (32) stressed that untoward reactions to drugs may be due to genetically determined factors such as the absence or deficiency of particular enzymes or the abnormality of a receptor Such an inborn error of metabolism" may be entirely innocuous for the individuals unless a particular drug is prescribed It is an important aim for future research to find the inborn error of metabolism underlying the drug-induced anaphylactoid syndrome

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VARIATIONS IN L DOPA ABSORPTION

Report of a Parkinso ian Patient with H gh Plasma Dopa Conc tration after Therapeutical L-d pa Dose

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Abstract In parkinsonian patient with weak effect of L-dopa on the parkinsonian symptoms, unusually high plasma levels of dopa and dopamine sulphate were noticed. The plasma concentration of tyrosine and phenylalanine were low during the whole observation period. The urinary excretion of dopamine sulphate and the ratio of dopamine sulphate to dopamine in urine ere high The reason for this high ratio of dopamine sulphate to dopamine could not be determined. The disappearanc of L-dopa from plasma after ad-ministration as only slightly reduced It was concluded that the L-dopa absorption this patient wa very efficient due to low dopa decarboxylation I the intestinal organs in spite of normal decarbo ylase activity in other peripheral tissues Such selective de crease in the dopa decarboxylation in the intestinal organs is presumably unusual, but illustrates that very marked individual variations in the L-dopa metabolism may occur in parkinsonian patients

The therapeutical dose of L-dopa varies ind vid ually n patients with Parkinson s disease ou patient material between 1 and 8 g/day (3) anc is not directly related to age body weight. ymp-toms or duration of the disease (20) The plasma level of dopa varies considerably after the same oral dose in different patients (1 7 8 11 12 14 1 24 26 27 28) In relation to the adm nistered dose the plasma level of dopa has been found to be low due to the fact that L-dopa is to a large extent decarbo ylated already i the intestinal organs (1 5 10, 18) Furthermore the turnover rate of absorbed L-dopa is rapid (18)

We have howev found one patient who had a very high plasma level of dopa on ordinary therapeutical dose The studies performed i this patient indicated a low rate of dopa decarboxyla-tion in the gastrointestinal organs but normal decarboxylation in othe peripheral ti sues cluding the kidneys.

CASE REPORT

The patient was a woman aged 76 years at the of this study At the age of 57 she had had ympt of encephalomeningitis. Three years later th fi st symptoms of Parkinson disease appeared When he parkinsonian ymptoms had persisted for fiv years thorough neurological investigation as made which confirmed the diagnosis On thi occasion the cerebro-spinal fluid w examined and EEG and pneumoence phalography ere performed with normal findings Anticholinergic therapy was then started with certain effect at th beginning of the treatment but later the parkinsonian symptoms continuously increased I 1969 the patient had a ery advanced physical handicap and she needed assistance in most of the activities of daily living (ADL group 4) (20).

In 1969 before the start of L-dopa treatment, the cerebrospinal fluid was examined The homovanillic acid (HVA) concentration as 6. µg/l which is value similar to that found in control material the value was higher than in most parkinsonian pa-tient (19)

During the time of the study the patient had no signs of cardiac insufficiency and X-ray of the heart and lungs was normal. During the time of the L-dopa treat ment (18 months) her body weight varied between 50 and 52 kg. She did not have any history of renal dis-ease but the serum creatinine was 1.5 mg/100 ml (re ference value 0 6–1..) and the glomerular filtration 41 ml/min as determined by the method of Alestig et al (2) Urinary tests and routine haematological tests as well as liver function tests were normal.

L-dopa treatment was started in 1969 when the pa-tient was 75 years old. At the maximal tolerable L-dopa dose of 3.0 g/day small effect on hypokinesia and rigidity could be seen, but the patient still belonged

one. In our patient not only the excretion of dopamine sulphate but also the ratio conjugated to non-conjugated dopamine was extremely high. This is a remarkable observation since after i.v. administration of L-dopa only about 6% seems to be excreted as dopamine sulphate, whereas as much as 17% is excreted in free form (15) resulting in a ratio of about 0.4 between dopamine sulphate and free dopamine in urine. The ratio of conjugated to non-conjugated dopamine could therefore be expected to be low in a patient with an efficient L-dopa absorption and not high as observed in the present case. We do not know the reason for this high degree of dopamine conjugation in our patient.

The total excretion of dopamine and dopamine sulphate in urine was also large in relation to the oral L-dopa dose given. This shows that L-dopa was readily decarboxylated once it had reached the general circulation. It might also indicate that the further degradation to HVA, which is the main metabolite, was depressed. Unfortunately determination of the HVA excretion was not possible because the urine samples available contained salicylate which interfered with the determination.

The somewhat lower elimination rate of i.v. administered L-dopa in this patient compared to the parkinsonian patients is in agreement with the assumption that there was a preferential inhibition of the dopa decarboxylation in the intestinal organs but a normal decarboxylation in other organs such as brain and kidney. The reason for such a selective inhibition is not known. A marked inhibition of the decarboxylase activity in the liver of rats has been observed (13) after chronic administration of L-dopa. However, we have recently studied the degree of dopa decarboxylation in patients treated with L-dopa during 6–24 months without being able to demonstrate a reduced rate of dopa decarboxylation in the gastrointestinal organs (18).

L-dopa treatment has been reported not to influence the levels of the other plasma amino acids (1, 22). In the present patient the levels of tyrosine and phenylalanine, however, were constantly low. This might only partly be the result of a low protein intake, as the levels of the other amino acids were normal. The low tyrosine and phenylalanine concentrations might also be an effect of the unusually high plasma dopa level influencing the homeostasis mechanisms, or an effect of the high intestinal L-dopa absorption, resulting in a competitive inhibition of the absorption of structurally related amino acids. There appeared not to be a primary defect in the absorption of free phenylalanine as judged from loadings with this amino acid.

The conclusion is that in this case the majority of orally administered L-dopa was decarboxylated in the kidneys and not, as previosly demonstrated in other patients, mainly in the gastrointestinal tract. The reason for the high dopamine sulphate production in our patient could not be determined. To what extent this anomaly in the dopa metabolism has influenced her clinical condition and the therapeutical response is not known. The present study shows that a conventional treatment of a patient with a classical Parkinson's syndrome can result in an extremely high dopa concentration in plasma and that marked variations in the L-dopa metabolism may occur.

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LACTIC ACIDOSIS IN EPINEPHRINE POISONING

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Abstract Lactic acidosis as result of epinephrine poisoning is discussed A case report illustrates the clinical condition after very large dosage of epinephrine (20 mg i.). The pathogenesis of lactic acidosis in this situation is discussed and suggested to be of type II A and II B according to Huckabee W advocate the treatment with insulin glucose and bicarbonate

Lactic acidosis was first mentioned in 19_5 in dehydrated children (?) but this work did not provoke any investigation of the etiology of lactic acidosis until Huckabee (5) in 1961 analysed the causes of elevated lactate concentrations in the blood Recently Oliva (8) has published a review on the subject We ha e observed an unusual cause of lactic acidosis—epinephrine poisoning—and we here discuss the possible mechanism of lactic acidosis on the basis of the present case.

CASE REPORT

A _3-year-old male know to be morphine addict had injected ml Hectalin =70 mg epinephrine (verified by the police and the patient) in an attempt at suicide 6 hours prior to arrival in the Intensive Care Unit. Initially he was somnolent with widely dilated pupils non-reactive to light, enormously constricted peripherally with cyanosis. Respiration wa accentuated—Kussmaul-like —and tachycardia (120 beats/min) was present BP 125/75 mmHg. ECG showed sinus tachycardia and ischemic changes in all leads Gradually he became comatose and blood chemistry showed a heavy metabolic acidosis (Fig 1) Ketonuria was absent judged by the Acetest BUN was normal

Treatment was started with oxygen administered through an endotracheal tube, 200 mEq bicarbonate in 4 hours, 70 IU crystalline insulin and 1 700 ml isotonic glucose Potassium supplement 50 mEq w given Fou hours after arrival the patient developed pulmonary edema and hypotension After successful treatment with 0.50 mg ouabain 100 ml 20% human albumin and 80 mg furosemide hi condition rapidly improved acidosis abolished and lectrolytes became normalized 7 hours after arrival (T ble 1) The patient as dismissed fully wake for further supportive treatment in the Psychiatric Department

Laborat ry alue

Hb 1_9 g/100 ml hematocrit 39 0% pO_2 75 mmHg arterial pH 7 07 p_aCO_2 _3 mmHg, tandard bicarbonate 9_ mEq/l serum sodium 135 mEq/l, serum potassium _6 mEq/l BUN 14 mg/100 ml serum bilirubin 0 5 mg/100 ml, *prothrombin time* 33% *SGOT* 65 U/l *normal alue* <40) plasma glucose 379 mg/100 ml, serum lactate 5 5 mEq/l (normal value 0 55–1 75) serum methanol 0 No acetone blood glucose or albumin in the urine

ECG showed sinus tachycardia with ischemic changes in all leads and X-ray thora revealed pulmonary edema

DISCUSSION

Lactic acidosis i metabolic acidosis characterized by a significant elevation of lactate concentration in the blood (4 8) Clinically the cond tion is dominated by Kussmaul respiration and development of shock and coma (1 8 9 11)

H ckabee (5) was the first to suggest classification of lactic acidosis nto two main types T pe I is characterized by an elev tion f both lact and pyruvic acid so that the lactat pyru te rat o is constantly 10 1 I thus type ther is no x es lactate In type II the lactate concentration is augmented without corresponding elevation of pyruvic acid the lactate pyru at tio w ll be increased and xces lactate w ll be present Th is often seen when hypoxia and/or circulatory fail ure dominate the cl nical picture Thi type ha bee subclassified as II A in which tis ue hypoxi and desaturation of art rial bl od are prese t and a II B in which no obvious igns of tis ue h poxia are present The last mentioned condition is often secondary to diseases with disturbed lactat elimination, renal and hepatic insufficiency

Greene (3) found lactate le els f 1 3– 9 mEq/l follow ng epinephrine injections of 3 µg/kg n 70 mi

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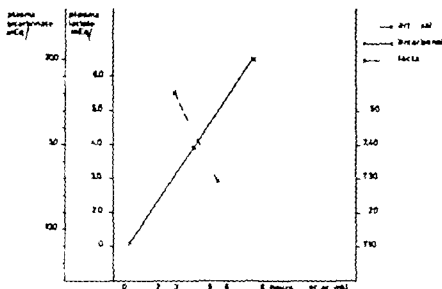


Fig. 1. Plasma lactate (– –), plasma bicarbonate (—) and arterial pH ( ) during treatment with glucose, insulin and bicarbonate.

in healthy human volunteers. We have found no investigation of the effect of very large doses of i.v. epinephrine on human subjects. Primarily epinephrine will stimulate the glycogenolysis, resulting in augmented glycolysis (10). This will result in an increased lactate–pyruvate concentration, but the lactate pyruvate ratio will be constant 10 : 1 (1). If large doses are given, the peripheral action of epinephrine will dominate, resulting in tissue hypoxia and lactic acidosis of type II will be produced. The decreased perfusion of liver and kidney (6) will further accentuate the acidosis, as the lactate elimination is greatly diminished. We suggest that the lactic acidosis in the present case was a combination of types II A and II B.

We treated the patient with insulin, glucose and bicarbonate (7). The only complication seen was acute forward failure, probably caused by the ischemic strain on the heart. As epinephrine is rapidly inactivated from the body, the treatment of choice in poisoning resulting in lactic acidosis will generally be supportive in regard to respiration and circulation. We do not find treatment with peritoneal or hemodialysis indicated in cases of lactic acidosis when the precipitating factor is known and no longer present.

Table I. *Clinical signs observed in the patient during the first 7 hours after admission*

+ = present – = absent

	Hours						
	0–1	1–2	3	3–4	4–5	5–6	6–7
Polypnea	+		+		+		–
Coma	–	+		+	( )	–	–
Peripheral constriction	+				(+)	–	–
ECG ischemia	+					–	
BP	→	→	↓	↓	↓	→	→
Pulmonary edema	–	–	–			+	–
Acidosis	+++			+		–	–

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FATAL POISONING WITH DEXTROPROPOXYPHENE-CONTAINING ANALGETICS–SUICIDE OR NOT?

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Abstract Among 63 cases of fatal poisoning with analgetics containing dextropropoxyphene alone or in combination with ethyl alcohol and/or other drugs suicide could be proved in only 20 cases In a consecutive group of 63 cases of fatal poisoning with barbiturates 42 were regarded as suicide. Thus the number of other deaths turned out to be much higher in the dextropropoxyphene series. The uncharacteristic clinical course and the absence of specific pathoanatomical findings at autopsy mean that such intoxications can easily be overlooked.

Analgetics containing dextropropoxyphene were introduced in the middle of the 1950s and ha e since been widely used and prescribed not only by physicians but also by dentists In Sweden pure dextropropoxyphene chloride is a component of the compounds Dolotard and Doloxene® It is combined with salicylic acid caffeine and phenacetin in capsules (Doloxene comp N and comp forte N) T Doleron® contains also transergan (2 diethylaminoethyl-phenothiazine-carboxylate chloride) and phenacetin has been replaced by phenazone T Doleron natt® contains not only the substances in Doleron® but also a barbiturate vinbarbital (5-ethyl 1 methyl-butenyl-barbiturate) instead of caffeine Other dextropropoxyphene preparations are combined with salicylic amide (T Dolomid forte®), chlorzoxazone + salicylic acid (T Paraflex comp N®), paracetamol (T Distalgesic®) phenaglycodol + salicylic acid (Caps. Vondar®) phenprobamate + salicylic acid (T Tergoflex®) and in the latter preparation also pentobarbital (T Tergmox®).

Recent years have w tnessed an increase the frequency of fatal poisoning with such analgetics often combined with ethyl alcohol or barbiturates In a strikingly large percentage of these cases it could not be proved that overdosage occurred with suicidal intent, such as is often the case in fatal poisoning with soporifics To further evaluate this impression a consecutive series of 63 such cases of fatal poisoning with dextropropoxyphene-containing analgetics was studied regarding the mode of death and compared with an equal number of cases of poisoning with barbituric acid

MATERIAL AND METHODS

Analgetic containing dextropropoxyphene (series I)

The material consisted of 63 cases examined post mortem between Feb. 1971 and Jan. 1974 The age and sex distribution is given in Fig 1 Three of them were cases of fatal poisoning with Doleron (dextropropoxyphene chloride phenazone caffeine acetylsalicylic acid transergan) alone and 60 with combinations of analgetics containing dextropropoxyphene and barbiturates (e.g. T Doleron natt®) or alcohol or psychopharmacological drugs

Only cases fulfilling one or more of the following criteria were regarded as suicide 1) Discovery of suicidal note written by the deceased 2) Pronounced aversion to living or know depression. 3) Earlier attempted suicide 4) Examination revealing large amounts of the toxic substances in various organs I cases in which it could not be decided whether death had been suicidal or not, the relatives were contacted later N new information confirming suspected suicide was ever obtained.

The chemical analyses were performed in the Government Laboratory for Forensic Chemistry Stockholm The following method were used. The liver tissue was homogenized and extracted with ethyl alcohol the extract containing about 70% ethyl alcohol Extract of ethyl alcohol in an amount corresponding to 10 g liver tissue was purified according t the cooling method (1).

Dextropropoxyphene and other substances, for example salicylic acid phenazone and barbiturate derivatives,

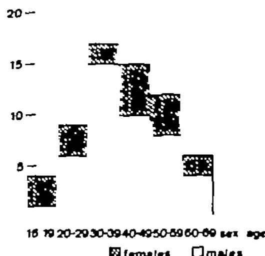


Fig. 1. Age and sex distribution of the dextropropoxy phene material.

were extracted with chloroform from weak acid solution (pH ~3) in the form of the sol ble hydrochloride. The chloroform xtract was analysed for acid compound (UV spectrophotometry paper chromatography), and neutral compound (UV spectrophotometry thin-layer chromatography and colour reaction). Dextropropoxy phene analyses were mainly performed by ga chroma tography. The glass column were filled with one of the ymers SE 30 Hi-Eff and OV 17 as stationary phases and placed on carrier material of chromosorb and/or gaschrome-Q-type whereafter the substance eluted could be measured. The identity of the dextropropoxyphene peak was erified by mass spectrometry. For quantitative calculations the gas chromatography peak in liver extract corresponding to 4 mg liver was compared with the peak of 0... µg dextropropo yphene hydrochloride standard substance (2).

Barbituric acid poisoning (series II)

The consecutive series consisted also of 63 cases analysed in the same fashion as in series I. In these cases the relatives were not contacted anew. The analysis included xamination for ethyl alcohol barbiturates, meprobamate metaqualone benzodiazepine derivatives, hydroxyzine hexapropymate phenothiazine derivatives and tricyclic amines.

RESULTS

Analgetics containing dextropropoxyphene

In only ?0 of the 63 cases had investigation by the police given reason to suspect intentional poisoning. In all the other cases the police had requested postmortem examination because death had been sudden and the cause obscure. In these cases the physicians were not prepared to issue a death certificate on the basis of the results of the investigation by the police knowledge of prior illnesses of the deceased and/or external inspec tion of the corpse. Postmortem examination had not revealed any morphologically clear evidence of the cause of death in these latter cases either. Tablets containing dextropropoxyphene are often completely dissolved in the stomach for which reason the absence of fragments of such tablets does not exclude the possibility of poisoning by such substances. For this reason chemical analyses were carried out by the Government Laboratory for Forensic Chemistry.

Table I. *Patient data of 4 cases in series I estimated drug consumption and concentrations of substances in liver tissue and ethyl alcohol in the blood*

BARB = vinbarbital. SAL = salicylic acid. PHEN = phenazone. DPO = dextropropoxyphene. ETH = ethyl alcohol

Sex	Age (y)	Interval between last seen living and found dead	Drug consumption according to police investigation	Concentrations of substances in liver tissue (mg/100 ml) and ETH in blood (‰)				
				BARB	SAL	PHEN	DPO	ETH
♂	32	3 d.	2 Doleron +23 Doleron natt*	1.6	1.6	0.7	0.7	–
♀	42	1 d.	23 Doleron natt + 9 Lunedon*	1.3	5.6	6.7	1.8	–
♂	36	2 h	30 Doleron natt	1.4	1.5	6.2	3.0	1
♂	32	3 h	L. Doleron natt	0.9	1.6	3.0	.0	1.4

Table II. *Division into groups according to substances found in liver tissue and ethyl alcohol in blood and number of suicides or other deaths (accidental intentional or natural)*

Group	Suicides	Other deaths
1 DPO-SAL-PHEN	3	0
2 DPO-SAL-PHEN-BARB	6	6
3 DPO-SAL-PHEN + ETH in blood	2	1
4 DPO-SAL PHEN-BARN + ETH in blood	2	18
5 Different combinations of DPO-SAL PHEN + ETH + some of the following: meprobamate benzodiazepines barbiturates, methaqualone, chlorzoxazone hexapropymatum and tricyclic amines	7	7
	20	43

Abbreviations as in Table I.

The chemical analyses were generally performed on liver tissue. The concentrations of the substances found varied considerably: phenazone 1 1–15 1 salicylic acid 0.5–35 dextropropoxyphene 0.2–28 7 and barbiturates of vinbarbital type 0.28–7.3 mg/100 g. A Doleron® tablet contains 65 mg dextropropoxyphene chloride 350 mg acetylsalicylic acid 150 mg phenazone 50 mg caffeine and 5 mg transergan. (No method is available for demonstrating transergan) T Doleron natt® also contains 50 mg vinbarbital instead of caffeine No certain relation was found between the amount of drug taken when known and the tissue concentration of the substance by chemical analysis This may to certain extent be due to different durations of the periods between death and post mortem examination, and it could therefore not be determined how much of the substances had been excreted or metabolized (Table I) Neither was the duration of survival after ingestion of the drug known It is also remarkable that no correlation was found between the concentration of the substance in the liver tissue and the relative amounts of the substances in the tablet

The material was divided into fi groups according to the substances found by chemical analysis (Table II)

Of the total number of cases the dextropropoxyphene series 20 (about 3 %) were classified as suicide In 13 of these cases it was clear from the preliminary police reports that poisoning had been suspected.

In group 1 (age range 19–20 years) i.e cases of poisoning with pure Doleron® all deaths were judged to be suicides and witnesses had seen one patient take a handful of Doleron® tablets The concentrations of the components in the liver were as follows. dextropropoxyphene .8–28 7 salicylic acid 3.8–35 and phenazone 6 0–15 1 mg/100 g.

As regards group 2, according to the investigation by the police a varying number of different packets of tablets containing soporifics psychopharmacological drugs and analgetics were found at discovery of the deceased But containers of T Doleron natt® were found only in 6 of the 1 cases In this group the concentrations of the substances in the liver varied as follows vinbarbital from 1 1 to 3 7 mg/100 g, salicylic acid 1 4–9 8 phenazone 1 9–13 0 and dextropropoxyphene 0 3–1_0 mg/100 g.

For group 3 analgetics containing dextropropoxyphene + ethyl alcohol concentrations of the components in the liver and of ethyl alcohol in the blood are given in Table III.

One of the cases in this group was remarkable While under the influence of alcohol a 77 year-old chronic alcoholic (the last case in Table III) had taken _5 Dolotard capsules. One capsule contains 0 15 g dextropropoxyphene chloride He died

Table III *Liver eights and oncentrations of subst es in liver tissue in group 3*

Sex	Age (y)	Liver wt. (g)	Concentrations of substances in liver tissue (mg/100 ml) and ETH in blood (‰) DPO	SAL	PHEN	ETH
♂	36	190	0.3	5 0	3 3	1 7
♂	41	1 920	0 5	0 5	1 1	9
♀	46	1 670	6 0		5 6	1 0
♂	35	1 510	1 7	7	0	1 9
♂	61	1 800	11	8	4 9	1 9
♂	53		1	0 9	8 0	1 7
♂	47	1 710	0 3	1	1 9	1 3
♂	43	1 900	0 6	_7	3 7	1 4
♂	57	1 690	1 8		1 3	1
♂	4	2 140	1	10	3	0 6
♂	61	1 790	0 4	15	14	4
♀	16	1 430	7.0	4 0	0	0 6
♂	28	_30	1.9	6	3 0	1 9
♂	77	830				0 9

Abbreviation as in Table I

six hours later Chemical analysis by the Government Laboratory for Forensic Chemistry revealed only dextropropoxyphene and ethyl alcohol The autopsy revealed no pathological changes in the internal organs beside pulmonary oedema. As far as is known this is the first case of fatal poisoning with pure dextropropoxyphene chloride and ethyl alcohol on record

Regarding group 4 analgetics containing dextropropoxyphene and barbiturate of vinbarbital type + ethyl alcohol packets of Doleron natt® tablets were found in 9 cases In this group the concentrations of the substances in the liver were as follows dextropropoxyphene 0.2–17 7 salicylic acid 1 1–11 3 phenazone 1 3–9 1 and barbiturate of vinbarbital type 0.28–7.3 mg/100 g. The concentration of ethyl alcohol in the blood ranged between 0.3 and 2 4 ‰

Pulmonary oedema was revealed in all cases at autopsy No specific pathoanatomical changes were demonstrable Fragments of the tablets were found in the stomach in 29 cases including 15 with the afore mentioned combined poisoning with other tablets

Barbituric acid poisoning

In this series 42 (about 67%) of altogether 63 cases were judged to be suicidal Combined intoxication with ethyl alcohol was noted in 32 cases In of the cases of suicide and in 14 of the obscure ses the fatal poisoning was also accompanied by ethyl alcohol

DISCUSSION

Drugs containing dextropropoxyphene and available in Sweden are described in the introduction Inquiry among the pharmaceutical manufacturers revealed that in Sweden (about 8 mill inhabitants) at least 90 mill tablets of these preparations were sold in 1973 This illustrates the present extensive use of drugs containing dextropropoxyphene The risk of poisoning is therefore considerable For comparison it might be mentioned that the corresponding figure for soporific tablets containing barbituric acids was at least 60 mill The number of cases of fatal poisoning with these preparations examined post mortem in the Department of Forensic Medicine Lund in 1973 was 101 the corresponding figure for cases of poisoning with dextropropoxyphene-containing analgetics during the same period was 3

The first case of fatal poisoning with pure propoxyphene hydrochloride was described in 1964 (6) Since then several cases of fatal poisoning with compounds containing propoxyphene and dextropropoxyphene have been reported (7 8 9 10) At autopsy only pulmonary and possibly cerebral oedema was demonstrated The clinical course has been described as deep coma tendency to convulsions and inhibition of the respiratory and circulatory centers (5) One case has been reported with repeated respiratory arrest alternating with wakefulness (4)

Analysis of the above material showed that poisoning by drugs containing only dextropropoxyphene salicylic acid phenazone and transergan is only rarely fatal In all 3 cases in the present material such fatal poisoning was always regarded as suicide In the remaining 60 cases the fatal issue was ascribed to a combination of the above mentioned substances with either barbiturates with or without ethyl alcohol or a combination of psychopharmacological drugs

It cannot of course be excluded that additional substances not demonstrable by the analytical methods used had been taken by the deceased

The question is whether any existing disease might have contributed to the fatal issue in the cases observed In only two of the cases did autopsy which was mostly supplemented by microscopic examination reveal a serious disease which might have influenced the course of events One of these was a 43-year-old male diabetic and the other a 56-year-old man with cardiac hypertrophy In the former case autopsy showed mild myocardial fibrosis and moderate fatty degeneration of the liver in the second case left ventricular hypertrophy with a heart weight of 550 g and bilateral renal cysts In three additional cases autopsy revealed advanced arteriosclerotic changes in the coronaries and in one case diffuse myocardial fibrosis No signs of recent ischaemic myocardial lesions were demonstrable in these cases at microscopic examination with Mallory PTAH staining. In five further cases there was fatty degeneration of the liver possibly owing to chronic abuse of ethyl alcohol

Perusal of the police reports available and the hospital records did not reveal any cases in which analgetics containing dextropropoxyphene had

been prescribed because the patient was suffering from some disease which might have been of importance for the fatal outcome In 36 cases the preparation had been prescribed for the deceased himself and in 6 for relatives or friends. In the other I cases it is not known how the deceased had come by the drugs

The course of poisoning with dextropropoxy phene-containing analgetics combined with alcohol and/or barbiturates psychopharmacological drugs, etc is very uncharacteristic as is apparent from the following observations

While eating a meal a 41-year-old man who had been drunk almost every day for some months, swallowed a handful of tablets in the presence of witnesses. He then fell asleep and four hours later he was found dead

A 28-year-old man had taken an unknown number of tablets while under the influence of alcohol He afterwards felt ill and ordered a taxi to a hospital. He had got out of the taxi but could not pay the fare After consultation with the personnel at the hospital he returned home in the same taxi to fetch the money He lost consciousness on the way home and died 1 hour later

At about 8 p m a 32 year-old man known to have abused drugs and alcohol was taken into custody by the police because under the influence of alcohol At the police station he was deprived of all his tablets It was found that he had escaped from prison. Therefore, at 2 a.m by which time he had sobered up he was returned to the prison All his possessions and clothes were taken from him and he was given prison clothes instead At 8 a.m. in the morning he was found dead This person had thus not had the opportunity to take any tablets after he had been caught.

It is remarkable that, after having taken dextropropoxyphene-containing analgetics together with alcohol the patients need only show signs similar to mild alcoholic intoxication and they may suddenly lose consciousnes and die soon there after It is not know whethe this delayed effect can be ascribed to transergan

Autopsy revealed remna ts of tablets in the stomach in 29 cases (ncluding 15 with combined intoxication) but not in the remaining 34 cases, though death apparently occurred relatively soon after ingestion of the tablets The lack of anatomical changes of poisoning nd the uncharacteristic course of poisoning clearly show how easily such intoxication can go unnoticed during the police investigation and autopsy This holds especially for cases with demonstrated pathological cardiac changes which could by themsel es explain a sudden death.

It is also interesting to note that the inter individual differences in the relation of the amounts of salicylic acid phenazone dextropropoxyphene and barbiturates in the liver bore no constant relation to the amount of ingested substances This has been pointed out earlier by Bonnichsen and Sköld (3)

As noted only 20 of the 63 cases of fatal intoxication could be classified as suicidal In the other cases the cause of death must be regarded as obscure Whether the deaths in these obscure cases were accidental is difficult to determine The tablets might nevertheless have been taken n an attempt to commit suicide The deaths might also be explained by lack of respect for or knowledge of the effects of the drugs or lack of judgement while under the influence of alcohol It is well known that alcoholics are prone to intensify the effect of alcohol by taking soporifics or psychopharmacological drugs. Moreover the tablets may have been taken not to commit suicide but to attract attention or arouse pity by placing the de ceased in a situation call ng for help and relief in order to obtain a solution to personal problems As for soporifics the risk of overdosage is probably generally known also to those who combine the consumption of alcohol with soporifics But when it comes to preparations described as pain killing" muscle relaxing or night analgetics one can hardly expect the laity to know anything about the risk of overdosage when combined w th alcohol and/or soporifics This may explain why fatal poisoning with dextropropo yphene-containing analgetics together with alcohol or soporifics must be onsidered to be uncertain as regards suicidal intent or accident in contrast to poisoning with barbiturates

In view of the above mentioned varying concentrations of the degradation products of dextropropoxyphene-containing preparations n the tissues caution must be exercised in the evaluation of suicide if only high concentrations of the substance are found in the organs of the deceased

It is well know that consumption of ethyl alcohol can have ynergistic effect on barbiturates One should suspect such ynergism in persons who

have consumed dextropropoxyphene-containing analgetics and ethyl alcohol or barbiturates. How ever a search of the literature failed to reveal any information on this assumption.

The frequency of such poisoning with combined dextropropoxyphene phenazone salicylic acid and barbiturates (e.g. Doleron natt[1]) suggests that it is not wise to prescribe large quantities of such drugs at one time. This applies in particular to persons who might be thought to be alcoholics or to abuse barbiturates or who have mental problems.

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HAEMODYNAMIC EFFECTS OF DIHYDROERGOTAMINE IN PATIENTS WITH POSTURAL HYPOTENSION

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Abstract. In four patients with postural hypotension the effect of i. and oral administration of dihydroergotamine (DHE) has been studied. The therapeutic effect of oral administration of DHE has also been compared to the effect of treatment with an antigravity suit. Heart rate intraarterial BP cardiac output (dye dilution technique) and central enous pressure were measured in supine position before and during simulated orthostatic stres induced by application of lower body negative pressure. I three patients forearm blood flow using venous occlusion plethysmography was also measured. Measurements were made before and after i. injection of DHE (0.01 mg/kg b. t.) The long-term effects of oral administration of DHE ere evaluated using an orthostatic test on tilt table before and during medication. Following i administration of DHE, systemic and central venous pressures increased in all cases. The effect on cardiac output was negligible and consequently the calculated total peripheral vascular resistance increased slightly in all cases. Forearm blood flow changed in proportion to cardiac output. Orthostatic tolerance increased in all cases. Following long-term oral administration an increased orthostatic tolerance was demonstrated in three of four cases. The effect wa comparable to that obtained with an antigravity suit. Thus acute and long-term administration of DHE can increase the tolerance to orthostatic stress in patients with severe postural hypotension. This is probably explained by effects on both capacitance and resistance vessels.

Postural hypotension is a rare disturbance of the reflex cardiovascular regulation of arterial blood pressure (BP) and heart rate (HR) (5 6 15). The most apparent and easily demonstrated manifestation of postural hypotension is a marked fall in arterial BP with no or at best only a slight increase in HR on head-up tilting. Though rare these patients present a great therapeutic problem (2, 11 14) Concerning therapy three main approaches are usually considered. measures aiming t 1) an increase of the blood olume) an increase of the peripheral ascular resistance 3) a reduction of venous pooling in upright position Thus mineral corticosteroids are supposed to operate mainly by increasing the blood volume while sympathicomimetic drugs increase the peripheral vascular resistance Antigravity suits re duce pooling of blood n the lower body on transition from lying to upright position (16). Antigra ity suits are often effective but their usefulness is often limited by the inconvenience experienced by these mostly elderly patients However modern modified antigravity suits may be a valuable complement to the therapeutic arsenal as hown by Rosenhamer and Thorstrand (18)

In a recent study Nordenfelt and Mellander (13) demonstrated that, in patient with so-called sympathicotonic orthostatic eaction, dihydroergotamine (DHF) exerts its effect mainly through constriction of the capacitance vessels thereby reducing venous pooling without demonstrable effect on the resistance essels Thus DHF could be expected to act as pharmacological antigravity suit. The chemically related non-hydrated alkaloid ergotamine tartrate was reported to be of value n controlling postural hypotension as early as in 1946 by Berfenstam (3)

The above mentioned observations moti ted the present tudy concerning the haemodynamic effects of DHE patients w th postural hypotension

MATERIAL

Four patients, one male and three females, with postural hypotension were studied. Their age varied be tween 71 and 81 years. All patients presented the

Table 1 *Effect of head-up tilting on blood pressure and heart rate in four cases with postural hypotension*

		Systolic BP (mmHg)		HR (beats min⁻¹)	
Case no	Tilt angle	Supine	Head-up tilted	Supine	Head-up tilted
1	90°	215	90	58	57
	80°	165	95	62	70
3	80°	105	Syncope	78	83
4	60°	155	85	90	87

typical picture of postural hypotension with prompt fall of arterial pressure and no or little increase in HR on head-up tilting Systolic BP decreased to below 100 mmHg and HR increased by les than 10 beats/min in all cases (Table 1)

CASE REPORTS

Cas 1

A 73-year-old woman Since 2 3 years dizziness and occasional fainting in the erect position During the last year unable to stand upright for more than a short time Antigravity suit tried with partial relief However the patient found the suit cumbersome and asked for alternative therapy No signs of cardiac decompensation ECG showed sinus rhythm and slight ST T changes. There was slight general cardiac enlargement.

C se 2

An 81-year-old woman Since many years attack of zziness and fatigue in the upright position Shortly before admission to hospital considerable deterioration. E sentiall normal ECG N cardiac enlargement and n signs of decompensation

C se 3

A 73-year-old woman. Since some months attacks of severe dizzines and occasional epileptic seizures for which she received antiepileptic medication Admitted to hospital because of these symptoms and difficulties in swallowing Investigation including oesophagoscopy revealed non-malignant constriction of the car dia region No signs of cardiac decompensation or cardiac enlargement ECG showed sinus rhythm and ST T depressions.

Case 4

A 71-year-old man. Typical ymptoms of postural hypotension since 7 years Initially good relief by the use of an antigravity suit. Deterioration since 3 years and during last year mostly bedridden On physical examination no signs of cardiac decompensation. ECG showed atrial fibrillation and ST–T depressions No cardiac enlargement.

METHODS AND EXPERIMENTAL PROCEDURE

Haemodynamic tudies after i administration of DHE

The patients received no premedication. All drugs used for treatment of their postural symptoms had earlier been withdrawn so as not to interfere with this investigation.

Intraarterial BP was measured through short teflon catheter inserted percutaneously into either brachial artery Central venous pressure was obtained by percutaneous introduction of a teflon catheter via an antecubital vein The position of the catheter tip was checked by fluoroscopy and/or by respiratory pressure changes typical of intrathoracic recordings. Intravascular pressures were measured using strain gauge transducers (Statham P23Db) and recorded on an ultraviolet recorder (ABEM Ultralette) The reference level for pressure recordings was the mid-thoracic level in the supine position. Mean pressures were obtained by electrical integration. HR was obtained from the ECG Cardiac output was determined by dye dilution technique using Indocyanine green as indicator (Beckman Cardiac densitometer) Forearm blood flow was measured using an air-filled plethysmograph according to Dohn, modified according to Graf and Westersten (10) A rough estimation of changes in venous tone was obtained by comparing the volume increment in a forearm segment caused by inflation of collecting cuff to 30 mmHg above the pressure necessary to cause a barely detectable volume change of the segment (9).

Recordings were first made during control condition in supine position and then approximately 3 min after application of negative pressure around the lower body (LBNP) During the LBNP procedure the lower body (below liac crests) was enclosed in an airtight box with the patient resting in horizontal position. The pressure within the bo was reduced with an electric pump. The whole procedure was then repeated 10 min after administration of 0.01 mg DHE/kg b. t. The LBNP was adjusted in every case so as to mimic the BP and HR reactions during the initial tilt test

Long-term ffect of oral administration of DHE

After withdrawal of all treatment for postural symptoms, BP and HR were recorded at rest in supine position and after head-up tilting to an angle of 60-90° The tilt angle had to be varied individually according t the severity of ymptoms. BP was measured using a sphygmomanometer cuff HR w s determined from the ECG This procedure w s repeated after application of an antigravity suit inflated to pressure of 40 mmHg The antigravity suit used was designed for medical use (Camp Scandina ia)

The patients were then reexamined with a similar tilt test 3-7 weeks after administration of a daily dose of 4-10 mg DHE In cases 1 and 2 the blood olume using the carbon monoxide rebreathing method (17), was determined before and after approximately 1 months treatment In order to avoid the effect of diurnal variation in orthostatic reaction all examinations

ere performed at approximately the same hour of the day

RESULTS

Circulatory effects of LBNP During LBNP systolic BP fell markedly in all cases—more than the diastolic pressure—and consequently the pulse pressure decreased (Fig 1) In case 3 the BP was higher in the control situation than during the initial tilt test, presumably because the patient was somewhat dehydrated during the initial test.

Central venous pressure and cardiac output decreased in all cases during LBNP (Fig.) The decrease in cardiac output was mainly caused by a marked decrease in stroke volume (on an average 38%). The calculated total peripheral vascular resistance decreased in case 1 and was relatively unchanged in the other patients

Forearm blood flow was measured in three cases It was unchanged in case 4 and somewhat decreased in cases and 3 (Fig. 2). Thus forearm blood flow did not show the marked decrease normally found during LBNP

Venous tone measured as venous volume as described under Methods showed no detecable change during LBNP

Effect of i administration of DHE During control condition DHE caused increases in systemic BP and central venous pressure in all cases (Fig.) Cardiac output was essentially unchanged after DHE and consequently the calculated total peripheral vascular resistance increased There was no consistent change in forearm blood flow

During LBNP arterial BP was maintained at a higher level following DHE (Fig. 2) In all cases cardiac output was somewhat larger during LBNP following administration of DHE HR increased in all cases after administration of DHE Stroke volume decreased much less during LBNP after administration of DHE (a reduction of 12% compared to 38%) The calculated total peripheral vascular resistance was higher in all cases The central venous pressure fell to about the same level as before DHE.

Forearm blood flow showed only small and insignificant changes V nous tone which increased after administration of DHE was not significantly changed during LBNP

Side-effects Except for case 1 who experienced a transient sensation of pressure in the fore

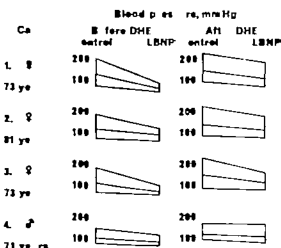


Fig 1 Effect of lower body negatv pressure (LBNP) on systemic BP before and after administration of 0 01 mg dihydroergotamine (DHE)/kg b wt in four cases with postural hypotension

head subsiding within 8 min there were no objective symptoms No ECG changes were bserved.

Effect of long-term oral administr tion of DHE The effect of DHE was compared with the effect of an inflated antigravity suit Each patient was examined with a tilt test with and without inflation of an antigravity suit. After more than 3 weeks oral therapy with 4–10 mg DHE daily a new tilt test was performed (Fig. 3) Without treatment all patients experienced severe dizziness during tilting. With an antigravity suit inflated to 40 mmHg BP was maintained at a higher level during the tilt test and the bove mentioned symptoms were partially relieved in all cases ex cept no 4 It may seem as if the difference with inflated antigravity uit, being no more than about 5 mmHg n two cases is relat ely modest. However this was sufficient to accomplish marked relief of symptoms

When the tilt test was repeated after oral administration of DHE arterial BP was maintained on the same average level as that obtained with an inflated antigravity suit. Patient 4 who had atrial fibrillation and a ery low cardiac output, could not be improved by either inflation of an antigravity suit or administration of DHE orally

Side-effects Patien 1 experienced slight gastrointestinal discomfort when receiving more than 4 mg DHE daily

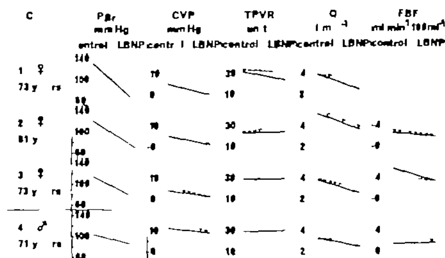


Fig 2 Effect of lower body negative pressure (LBNP) on some haemodynamic variables before (–) and after (– –) i administration of 0.01 mg dihydroergotamine (DHE)/kg b wt. in four cases with postural hypotension.

DISCUSSION

In order to obtain a constant reference level for intravascular pressures LBNP was chosen instead of the usual tilt test to achieve a blood volume displacement (1 8) This is especially important when measuring the low central venous pressure In each case the LBNP was adjusted so as to obtain approximately the same BP and HR responses as had been obtained during the initial tilt test

During LBNP cardiac output decreased with little or no change in forearm blood flow indicating a lack of reflex constriction of peripheral resistance vessels Deficient reflex control of resistance vessels is probably the most important factor for the inability of these patients to maintain normal BP during orthostatic stimulus

When analysing the effect of DHE on healthy normals and sympathicotonic orthostatic patients Nordenfelt and Mellander (12, 13) have shown that DHE exerts a selective and powerful constrictive effect on capacitance vessels without much effect on resistance vessels. In accordance with these authors we have found that DHE increases systemic BP and central venous pressure. This increase in central venous pressure was not accompanied by any change in heart volume after more than two months of oral therapy In the study by Nordenfelt and Mellander (13) on patients with sympathicotonic orthostatism the observed increase in cardiac output is sufficient to

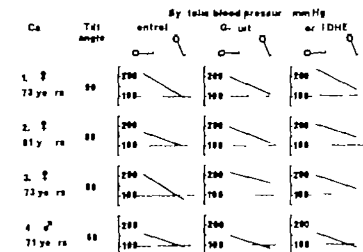


Fig 3 Effect of head-up tilting of systolic BP during control conditions after inflation of an antigravity suit and following more than three weeks of oral administration of dihydroergotamine (DHE)

explain the increase in BP. In the present cases with postural hypotension, i.e. defective sympathetic vasomotor control, cardiac output was essentially unchanged and thus the increase in BP must be explained by a constriction of resistance vessels. The explanation of this discrepancy considering the effect on the resistance vessels may be a difference in sympathetic nervous outflow to the vessels. Thus, during a blockade of sympathetic nerves as during spinal or epidural anaesthesia in man, a constrictive effect on the resistance vessels has been demonstrated (4, 7). Recent studies indicate that the constrictive effect is limited to parts deprived of their sympathetic tone (7). This is in agreement with studies by Mellander and Nordenfelt (12) who in cats showed a constrictive effect of DHE on resistance vessels only after sympathectomy.

However, the demonstrated increase in peripheral venous tone after administration of DHE helps to reduce venous pooling and the decrease of cardiac output during LBNP and thus contributes to maintain BP on a higher level.

The present results show that administration of DHE to patients with postural hypotension increases systemic BP and orthostatic tolerance. In the present small material the effect is well comparable to treatment with an antigravity suit. However, the increased orthostatic tolerance is obtained at the price of an increased systemic BP in recumbent position. This, however, probably applies also to most other forms of treatment.

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PRACTOLOL–PROPRANOLOL

A Comparison of Antihyperten i e Effect

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Abstract. The antihypertensive effect of practolol and propranolol have been compared in a double-blind cross-over study on 4 patients with mild to moderate hypertension. Practolol gave the same BP reduction th less reduction in heart rate.

It has been demonstrated that many β-adrenergic blocking drugs have BP-lowering action Propra nolol (Inderal®) is most widely used and effec tive (8 10 11 16) Propranolol appears to be a drug of comparable potency to bethanid ne guanethidine and methyldopa (10) The hypotensive action of propranolol is not quite clear but oral administration induces a fall in BP associated with a reduction of cardiac output. Total peripheral resistance is initially increased (6 14) Propranolol may provoke heart failure and bronchospasm in some patients. Increased peripheral resistance might explain clinically observed side-effects like Raynaud s phenomena and cold hands and feet. Disturbances of sleep indicate central nervous ac tion of the drug (10, 12, 16)

Practolol (Eraldin) is a β-adrenergic blocking drug which is relatively cardio-selective with much less effect than propranolol on the bronchial tree (9) or on peripheral vascular resistance (2 3) Practolol does not show the quinidine-lik effect of propranolol and affects cardiac output very little (2 7). The drug does not pass the blood brain barrier (1)

Practolol seems to reduce arterial BP (5 11 15) without increasing peripheral vascular resist ance From a theoretical point of view according ly the drug may be superior to propranolol n the treatment of hypertension with fewer expected side-effects

The aim of this study was to compare the BP lowering effect of practolol and propranolol as single drugs on previously untreated patient w th mild or moderate hypertension.

MATERIAL AND METHODS

Thirty previously untreated hypertensiv patients of average age 53 years (range 18), 27 men and 3 omen, entered the trial All patients were recruited from present population study and had casual BP more than 175 and/or 115 mmHg on t consecutive readings They were then followed until the BP stabilized after 3–6 out-patient controls All had benign essential hypertension. Patients with history of bronchospasm, cardiac failure or intermittent claudication were ex cluded. The female patients did not use oral contraceptives Six patients were excluded because of tablet failure

All patients were examined and followed in the Hypertension Clinic Sahlgren Hospital. The examination included ECG and X-ray of the chest, urinalyses of albumin and glucose serum creatinine uric acid and electrolytes. Eye-ground examination was done by the investigators BP was measured with mercury manometer (to the nearest 2 mmHg) in the right arm in the supine and standing positions after 5 min rest Diastolic BP (DBP)=phase 5 was used Heart rate (HR) was determined by pulse palpation With this examination 15 persons were classified as WHO stage I, while 9 showed signs of left ventricular hypertrophy or eye-ground changes as in WHO stage II

Design of the tudy

The antihypertensive effect of propranolol and practolol was evaluated in double-blind cross-over trial (Fig 1). In the first treatment period the pat ts were given either propranolol ($n=10$) or

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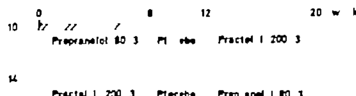


Fig 1 Design of the study

The doses of propranolol and practolol were chosen in the ratio 1/2.5 corresponding to the known β-receptor blocking effect of the two drugs (4). Thus propranolol was given in a dose of 40 mg three times daily for one week and then 80 mg three times daily for seven weeks. Practolol was given in a dose of 100 mg three times daily for one week and 200 mg three times daily for seven weeks. After another four weeks on placebo treatment those who had previously been on propranolol treatment were crossed over to practolol and vice versa. BP in the supine and standing positions and HR were measured at 0, 8, 12 and 20 weeks. At each visit a standardized questionnaire concerning side-effects was answered. After each treatment period the tablets were counted. Those who had taken less than 90% of the prescribed number were excluded.

RESULTS

Table I shows mean BP and HR before and after treatment for two months with propranolol and practolol for all 24 patients, 10 starting with propranolol and 14 with practolol.

1) Both drugs caused a significant reduction of BP and HR ($p<0.001$). 2) Both drugs caused an almost identical reduction of BP. 3) Propranolol lowered HR significantly more than practolol ($p<0.01$).

Fig. 2 presents the mean BP and HR before and after 8, 12 and 20 weeks treatment for those starting with propranolol and practolol, respectively.

1) Ten patients started with propranolol and 14 with practolol. No initial BP differences between the two groups were noted, but those in the practolol group had slightly higher mean BP values. 2) After 8 weeks of treatment those starting with practolol showed a significant BP reduction (SBP and DBP $p<0.001$). The smaller group ($n=10$) starting with propranolol did not show a significant BP reduction. No BP difference between the two groups was noted. Both propranolol ($p<0.001$) and practolol ($p<0.01$) lowered HR significantly, but propranolol somewhat more ($p<0.01$). 3) After 4 weeks of placebo treatment BP and HR rose towards pretreatment values. There was no statistical difference compared with initial SBP, DBP and HR except for DBP in the practolol group. 4) Eight weeks' treatment after cross-over produced almost identical BP reductions in the two groups. SBP and HR were significantly reduced ($p<0.001$) compared with pretreatment and placebo values for both groups. DBP was significantly reduced ($p<0.001$) for the propranolol group only.

Good BP control (SBP<160, DBP<100) was achieved with propranolol treatment in 19/24 patients and with practolol treatment in 17/24. Four of the patients were non-responders to both drugs.

Table II shows the effect of propranolol and practolol on 10 patients with DBP≥110 mmHg. Both drugs lowered BP significantly with no significant difference between the two groups.

Table I *Supine BP and HR for the total material before and during propranolol and practolol treatment and reductions of BP and HR* (Δ)

	Systolic BP (mmHg)			Diastolic BP (mmHg)			HR		
	$\bar{x}$		Δ	$\bar{x}$	$s_{\bar{x}}$	Δ	$\bar{x}$		Δ
Before treatment	173	17	–	106	7		82	13	
During propranolol treatment	156	20	17	96	10	10	63	10	19
During practolol treatment	155	18	18	97	8	9	69	10	13

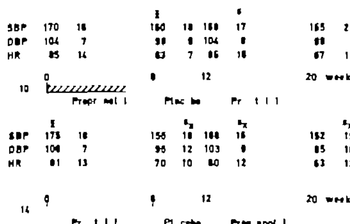


Fig 2 Supine systolic (*SBP*) and diastolic (*DBP*) BP and HR before and during propranolol and practolol treatment.

Side-effects

No patient developed signs or experienced symptoms of left ventricular failure or bronchial obstruction No sign of orthostatic hypotension was found. Neither interruption of treatment nor dose reduction were needed. The adverse effects were few and transient. On propranolol treatment one patient had sleep disturbances and two felt dryness of the mouth. One experienced initial vertigo and one had transient diarrhoea. On practolol treatment one patient felt malaise and two had cold extremities No significant changes in serum electrolytes creatinine or uric acid were noted No patient developed glucosuria or proteinuria.

DISCUSSION

The material n this study consisted of patients with mild to moderate hypertension The high drop-out rate might be explained by the somewhat strict criteria for cooperability

The design of the study was planned with an intermediate placebo period in order to have the patients back to their pretreatment BP levels HR returned to pretreatment values in both groups BP did not in those starting with practolol Although this might favour propranolol in the overall comparison, this slight difference should not change the conclusions drawn

The results showed that practolol seemed to have the same BP-lowering effect as propranolol in these patients with mild to moderate hypertension (Table I and Fig. 2) and even among those with BP above 110 mmHg (Table II) Only moderate BP reduction was achieved in contrast to other authors (5 11 15). These differences are probably explained by different compositions of the groups studied as well as differences in doses

Table II *Effect of 2 months practolol and propranolol treatm nt on BP nd HR for 10 subjects with untreated DBP≥110 mmHg*

	Untreated			After propranolol			After practolol		
	SBP	DBP	HR	SBP	DBP	HR	SBP	DBP	HR
	184	110	84	158	100	60	160	100	76
	162	110	76	134	94	66	142	90	68
	192	114	70	154	94	68	174	104	58
	174	116	100	154	98	56	148	100	78
	178	110	7?	140	90	58	144	96	64
	196	110	68	178	108	56	188	106	54
	200	110	60	192	11	56	182	106	66
	184	118	70	174	11..	60	170	104	56
	166	11	7?	154	98	58	146	98	64
	200	116	80	176	108	86	160	90	96
Mean	184	112	75	161	101	62	161	99	66
S.D.	13	3 1	10.9	18 1	8.0	9..	16.5	13	

administered The higher BP on practolol treatment is probably due to the intrinsic sympathicomimetic effect of practolol ( )

Fewer side-effects such as bradycardia, bronchial obstruction sleep disturbances or peripheral arterial insufficiency can be expected during treatment with practolol than with propranolol Side-effects however cannot be compared in this study because of the small number of patients

In mild and moderate hypertension practolol seems to have the same BP-lowering action as propranolol Whether fewer side-effects occur must be further investigated

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CHRONIC INTERSTITIAL PNEUMONITIS WITH FIBROSIS DURING LONG TERM TREATMENT WITH NITROFURANTOIN

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Abstract Chronic pulmonary lesions in conjunction with prolonged nitrofurantoin treatment ha occurred in 15 patients with ymptoms in the form of dyspnoea, cough fever and sometimes loss of weight The onset was insidious after 6 months to 6 years of continuou or intermittent treatment X-rays of the lungs showed streaky or patchy infiltration bilaterally most pronounced basally Spirometry revealed restrictiv ventilatory reduction in 8 of 10 patients. The time during which the patients continued with nitrofurantoin after the onset of ymptoms appears to be important but not decisive for the prognosis Observation of symptoms from the respiratory tract and regular X-rays during nitrofurantoin treatment ar recommended

Acute pulmonary hypersensitivity reactions during treatment with nitrofurantoin are now well known. The Swedish Adverse Drug Reaction Committee received more than 150 reports from 1966 to June 1973 The adverse reactions characteristically appear in acute form after 1–3 weeks of treatment—after a shorter time if the patient has received nitrofurantoin earlier The ymptoms consist of fever dyspnoea, leukocytosis and eosinophilia. More than half of the patie ts have cough In about 80 % of cases radiological lesions are seen, such as pulmonary infiltrates and pleural effusion The ymptoms recede within day or more after the cessation of treatment.

Reports of chronic nterstitial pneumonitis w th fibrosis are considerably less ommon. In 1966 the first report was published of a patient who after 1 year of treatment w th nitrofurantoin, reacted with severe dy pnoea and cyanosis (9). Since then another 1 cases of pulmonary reactions of chronic type ha e been reported (2 3 4 5 6 7 10 11) A this side-effect yet little know we considered it of nterest to publish the reports received by the Swedish Ad erse Drug Reaction Committee placed at our disposal by the reporting physicians

MATERIAL

The material comprises 15 reports of interstitial pneumonitis (ith fibrosis) received by the C mmittee from 1970 to Nov 1973 The causal connection with nitrofurantoin treatment has been judged to be probable or not excluded (1.).

RESULTS

Basic data concerning the patients pulmonary dis eases are presented in Table I Table II shows the nitrofurantoin dose the duration of treatment be fore and after the onset of symptoms cortisone treatment, if any converted to equivalent dose of prednisolone observation period after withdrawal of nitrofurantoin, and particulars of the subsequent course

Most patients were women aged 60–70 years who had been continuously or ntermittently treated w th nitrofurantoin over a lengthy period They do not appear to have been overdosed from the poi t of view of renal function Symptoms in the form f dyspnoea, cough fever and sometimes loss of weight, usually had an insidious onset after 6 months to 6 years of treatment In some cases the onset occurred i onjunction with cold I two cases the clinical symptoms were preceded by radiological disturbances In all patients X-ray of the chest showed pulmonary lesions i the form of streaky and patchy infiltrations bilaterally usually most pronounced basally On occasions there was also pleural effusion. In 8 of 10 patients spirometry revealed a restricti e entilatory reduc

Table I *Clinical data*

Medical record	
Solely urinary tract infection	7
Joint symptom	3
Joint symptoms + other disease	1
Other diseases	4
Lung X-ray	
Infiltrate	15
Infiltrate and pleural xudate	3
Spirometry	
Restrictive	8
Normal	
Not performed	5
Lung biopsy	
Fibrosis	4
Not performed	11
Serum reatinine (mg/100 ml)	
≤1..	7
>1..	
Not indicated	6

tion All patients had an elevated ESR in three cases more than 100 mm/h (Westergren) two had around 600 eosinophilic granulocytes/μl Serological reactions were recorded in several patients

Case 1

A 71-year-old w man, hospitalized 4 years earlier for hyperthyreosis and suspected collagenosis At the time of the present attack she had bee treated with Al- et (meth ldopa) for 1 year She fell ll with cough *in due ourse fever and dyspnoea X-ray of the* showed pleural fluid bilaterally and an abundance of distinctly delimited parenchymal infiltrates within large portions of the right and the basal portion of the left lung The ymptoms were new since her tay in hospital 4 years p eviously Spirometry revealed restrictive ventilatory reduction ESR 125 mm/h LDH 6.5 mU/l, ANF positive in dilutio 1/100 γ-globuli 2.5 g/100 ml, rheumatoid factor Coombs and Mantoux tests negative She wa not treated with corticosteroids She was asymptomatic 18 month after the withdrawal of nitrofurantoin the radiological ymptoms had regressed, spirometry showed normal conditions, ESR 60 mm/h, ANF 1/25 and LDH had become normalized.

Another two patients had a negative tuberculin test, six a rise of serum γ-globulin and two ANF above 1/100 In one patient (no 3) with positive LE cell phenomenon this persisted after the withdrawal of nitrofurantoin.

Case 3

A 71-year-old woman earlier generally healthy f ll ill with dyspnoea after a cold Her condition gradually deteriorated with increasing dyspnoea, cough and fever Erythromycin had no effect I the right lung X-ray showed xtensive diffuse hazy partially confluent infiltrates centrally and within most of the middle lobe around and connected with the hilar area. In the left lung there were thin hazy infiltrations outside the hilar area and basally LE cell ×3 were positive Mantoux negative She was treated with Betapred (betamethasone), 0.5 mg×3 and Tetralysal (lymecycline). At examination 6 weeks after withdrawal of nitrofurantoin she was asymptomatic and pulmonary X-ray showed almost complete recovery At regular check-ups during the following year the pulmonary picture was normal but she still had a few LE cells.

Liver reaction concurrently with pulmonary symptoms occurred in one patient (no 4)

C se 4

A 58-year-old man earlier generally healthy fell ill with jaundice without respiratory tract symptoms. Nitrofurantoin was withdrawn and the pathological liver function tests became normalized After 10 days nitrofurantoin was recommenced whereupon SGOT and SGPT rose again. After week of nitrofurantoin treatment he developed fever and catarrhal ymptoms. Pulmonary X-ray showed fairly xtensive streaky and patchy parenchymal infiltration basally bilaterally and minor infiltrations in the middle portions of the lungs. SGOT 570 mU/l, SGPT 410 He was not treated with corticosteroids T o months after the withdrawal of nitrofurantoin the pulmonary X-ray showed unchanged status. The liver function tests were normal At examination after 9 months he was subjectively healthy and the X-ray ymptoms had strikingly improved

Two other patients had somewhat elevated SGOT and SGPT values One patient (no 7) had had hepatitis of unknown genesis when the respiratory symptoms started At examination by reason of the pulmonary lesions 1 year later the SGOT and SGPT values were normal

Lung biopsy was performed on 4 patients The findings are illustrated by the following case

Case 5

A 61 year-old woman who had occasionally been troubled by a mild cardiac insufficiency and by ache and swelling *in hands and knees She was digitalized and used* salicylates regularly Fell ill with increasing dyspnoea followed later by fever and cough. V riou antibiotics were ithout effect. Pulmonary X-ray showed diffuse infiltrates bilaterally and spirometry restrictive vent latory reduction. Lung biopsy revealed considerable increase of interstitial connective tissue The contracted flattened alveoli were coated with slightly atypical dark-celled epithelium In the connective tissue there were few lymphocytes and occasional plasma cells. ANF was positive 1/128 She was not treated with corticosteroids At examination 18 months after withdrawal of nitro-

Table II *Nitrofurantoin dose and duration of treatment cortisone treatment observation period and course after withdrawal of nitrofurantoin*

Pat. no.	Age (y)	Sex	Nitro-furan-toin dose (mg)	Duration of treatment Before ymptoms	After symptoms	Predni-solone dose (mg)	Obs. period	Course Subjec tively	Radiol gical mp m
1	71	♀	50×3	11 mo.	mo	None	1 y 6 mo	Satisfactory	R
2	64	♀	5×	4 y	7 mo	5 3	7 mo	Satisfactory	R gres
3	71	♀		3 y 6 mo	4 mo	5 3	1 y	Satisfactory	Norm
4	58	♂	50×2	7½ mo	½ mo	None	9 mo	Satisfactory	Unchan d (mo
5	61	♀	?	Several y	5 mo.	None	1 y 6 mo	Improved	Regres
6	74	♀	50 3	At least 1 y	1 y 3 mo	None	1 y	Satisfactory	Regre si
7	67	♀	50×4–	6 mo	1 y	5	10 mo.	Improved	Some egressio
8	6	♀	?	6 y	4 y	5 4–	3 y	Death	Pr gressi
9	64	♀	?	Prolonged	mo	None	3 mo.	Improved	Regressi
10	54	♀	50 2	6 mo	6 mo.	5×3	mo	Improved	Unchanged
11	48	♂	50×	1 y 3 mo.	3 mo	None	5 mo	Satisfactory	Reg
12	69	♀	50×	1 y 3 mo	3 mo	None	½ mo	Improved	Regre
13	62	♀	?	4 y	3 y	Unknow	y 3 mo	Improved	Unch ed
14	69	♀	50 3–1	6 mo.	mo	40 1	6 mo	Improved	Regres
15	72	♂	50 3	2 y., 3 mo	3 mo	10 3	2 y 3 mo	Unimproved	U h ng

furantoin she was subjectively improved and the lung X ray as largely normal. Rheumatoid factor tests and tests for ANF ere negative.

Course

Nitrofurantoin treatment was discontinued in all cases Seven patients were given no further treatment. All improved subjectively and radiologically Eight patients received corticosteroids generally i low dosages Six of them improved but two had an unchanged radiological picture on follow-up after months and 1/4 years respectively Of the remaining two patients the condition of one (no 15) remained nchanged both subjectively and objectively while the other (no 8) died from respiratory and secondary circulatory in sufficiency

Case 8

A 6-year-old oman who had had dry bilateral pleuritis in youth. She fell ll with cough and in due course dyspnoea. Pulmonary X-ray showed bilaterally basally extensive streaky and patchy infiltrations with thinner portions, possibly emphysematous pockets Lung biopsy revealed fine bronchiectases, chronic bronchitis chronic pneumonitis and light interstitial fibrosis She was treated with Celestone (betamethasone), 0 5 mg 2–4 intermittently during the following 7 years, and with tuberculostatics The nitrofurantoin treatment was continued for at least 4 years af er the onset of symptoms During this time her cond ion aried subjecti ely but the radiological picutre remained much the same Thereafter both subjective and radiological impairment occurred and she died years after the onset of symptoms from respiratory insufficiency ith cardiac insufficien contributory cause Pulmonary X-ray just prior death had show pronounced streakines ithin t entire pulmonary parenchyma as pulmonary fi Spirometry revealed pronounced entilatory reduction restricti type Autopsy was not performed

The patients treated with steroid cont nued to receive nitrofuranto for on an average 14.8 months after the onset of symptom ompared with 6 months for those not given steroids

DISCUSSION

Chronic interstitial pulmonary lesions w th fibrosis may occu i several different conditions Hamman–Rich yndrome is accompanied by an acut or chronic diffuse interstitial pulmonary fibrosis The lesions commence with an interstitial oedema, exudate in the al eoli, and capillary damage An ncreasing fibrosis develops interstitially the alveol become coated w th a cubic epithelium which desquamates and the alveol are obliterated Dilation of the bronchioli may cause cysts and gi e the lungs honeycomblike appearance Lung function tudies show ventilatory redu tion of restricti e type and obstacles to diffusion. Radiological symptoms ppear only t a fairly late stage in the form of diffusely circumscribed dense fine patchines usually basally The cause of the Hamman–R yndrome i unclear I terstitial pneumoniti fibrosis similar to that i Hamman-Rich

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In the present material the prognosis was comparable to that reported in the literature Half of the patients had been treated with cortisone half had received no treatment. The prognosis was rather worse in the former group This is probably due not to the cortisone treatment per se but to the fact that the former group had been treated with nitrofurantoin for a lengthy period after the onset of symptoms and had more advanced pulmonary lesions.

CONCLUSION

From the material presented it is apparent that pulmonary lesions may be caused by current doses of nitrofurantoin. The prognosis seems to be worse the longer the patients have been treated after the onset of symptoms but there may be residual lesions even after brief treatment. Patients on prolonged or repeated nitrofurantoin treatment should be regularly checked for symptoms from the respiratory tract and for fever As the clinical symptoms may be preceded by radiological disturbances long X-rays should be checked say every six months

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has been elicited by busulfan, hydralazine and combined hydralazine and hexamethonium (1).

Collagenoses may cause similar lesions to those described by Hamman–Rich (8). In rheumatoid arthritis pleural exudate is the commonest pulmonary symptom. Diffuse interstitial fibrosis may also occur, appearing either before, concurrently with or after the joint symptoms. In fully developed systemic lupus erythematosus diffuse fibrosis is seldom seen, but pleural exudate is common.

The pulmonary lesions in sarcoidosis may develop into a chronic form with interstitial fibrosis. If one can follow the X-rays from the acute phase one sees how the hilar lymphoma diminishes in extent and the fine-patchy parenchymal infiltrates become increasingly coarse and confluent. At a later stage this leads to fibrosis and general emphysema (8).

Among the patients reported to the Adverse Drug Reaction Committee seven had no disease apart from infection of the urinary tract and had received no other drugs which might have a significance for the pulmonary lesions. These cases might possibly be assigned to the Hamman–Rich syndrome. The improvement which regularly occurred after cessation of the nitrofurantoin treatment suggests a causal connection between the pulmonary changes and this drug.

Of the other eight patients, cases 1, 5 and 13 had joint complaints before and/or during the disease; the rheumatoid factor test was not positive. [illegible] 1 and 5 received no corticosteroids. Nevertheless the ANF titre fell or became negative and the patients improved. In these cases nitrofurantoin must be considered to be the most probable aetiology of the pulmonary lesions (case 1 had at the same time been treated with Aldomet® (methyldopa) but had a negative Coombs' test). Case 13 had had pain in the joints for many years with slight swelling of the interphalangeal joints. Her lung disease improved subjectively but not radiologically during the cortisone treatment. This patient, however, had been on nitrofurantoin for 3 years after the onset of symptoms. It must be considered probable that the lengthy nitrofurantoin treatment caused the pulmonary symptoms.

Case 2 had an impaired renal function and secondary anaemia. She had received repeated blood transfusions and developed a sideroachrestic anaemia. Her pulmonary symptoms had from the outset been attributed to the uraemia, but on the one hand the serum creatinine was only moderately elevated (7.9 mg/100 ml) and on the other the radiological symptoms did not accord with those often found in uraemia (8).

Case 6 had a mild bronchial asthma and diabetes mellitus. She improved objectively under steroid treatment. In case 9 there was a cardial component which may have been a contributory factor. Case 10 had an apical tuberculosis, but the time relation with the nitrofurantoin treatment renders it probable that the latter was the cause of the lesions. Case 14 had joint symptoms and diabetes mellitus, which was treated with Diabinese (chlorpropamide) and Dibein® Retard® (phenformin). Here again there was a close relationship in time between the pulmonary symptoms and the nitrofurantoin treatment.

Twelve cases of chronic pulmonary reactions to nitrofurantoin have so far been reported in the literature (2, 3, 4, 5, 7, 9, 10, 11). One further case is to be published (6). The present cases accord with those previously reported in respect of nitrofurantoin dosage, duration of treatment before and after onset of symptoms, clinical picture, lung X-ray and spirometry findings. But in contradistinction to most of the previously reported cases the present patients usually had fever at the onset of symptoms.

As in the present cases, earlier published cases had a negative tuberculin test (4, 7, 9) and rise of γ-globulin (3, 7, 9, 11). Unfortunately LDH was investigated only in three of the present patients, one of whom had an elevated value. In the literature a rise of LDH is reported in four patients (5, 7).

The histological picture from lung biopsy (4 pats.) also accorded with reports in the literature (2, 3, 5, 7). Israel et al. (3) examined such lung tissue also in respect of antibody–antigen complex by immunofluorescence technique with negative result.

Most of the patients reported in the literature improved on cortisone treatment after the withdrawal of nitrofurantoin. One patient, however, who had received nitrofurantoin over a period of 6 years, 3 of which after the onset of symptoms, had unchanged lung function and radiological picture at follow-up. One patient, who had also received nitrofurantoin for several years after the onset of symptoms, died. This case has not yet been published (6).

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TRACE ELEMENTS IN SERUM AND URINE FROM HYPERTENSIVE PATIENTS TREATED FOR SIX MONTHS WITH CHLORTHALIDONE

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Abstract. Urinary excretion and serum concentration of antimony arsenic bromine cadmium, caesium, cobalt, copper gold, iron mercury molybdenum rubidium, scandium, silver tungsten and zinc from 6 hypertensive patients before and after 6 months treatment with chlorthalidone have been investigated. The method consisted in neutron activation analysis combined with recently developed ion exchange technique A previously observed increase in urinary excretion of bromine cadmium, cobalt, rubidium and zinc after short-term treatment with chlorthalidone was also present after long-term treatment with regard to rubidium and zinc and possibly also to bromine cadmium and cobalt. A significantly lowered concentration in serum after long-term treatment was found only for rubidium.

Earlier investigations (7 15 16) have indicated a possible connection between trace elements and arterial hypertension. In a previous study (19) the serum concentration and urinary excretion of 17 trace elements from hypertensive patients were found not to differ significantly from those of normotensive subjects However during treatment of the hypertensive patients with chlorthalidone (Hygroton®) a raised urinary excretion of bromine cadmium cobalt rubidium and zinc was observed The serum concentration after 5 days treatment was only decreased with respect to rubidium and the question arose whether the increased urinary excretion of these eleme ts was only a temporary phenomenon or whether it would continue during treatment and possibly cause a deficiency of some of the trace elements

The aim of the present tudy wa to investigate the serum concentration and the urinary excretion of ntimony arsenic bromine cadmium caesium, cobalt coppe gold iron mercury molybdenum rubidium, scandium sil er tungsten and zinc from hypertensive patients treated w th chlorthalidone for 6 months

MATERIAL AND METHODS

Six patients 4 men and 2 women ranging age from 28 to 60 years, with mild to moderat hypertension treated with chlorthalidone in daily dose of 50 mg per os for 6 months were subjected to the study BP on admission varied between 175/105 in the youngest patient and 260/150 in one of the women The heart sizes varied between 360 and 700 cm^3/m^2 and none of the patients had glomerular filtration rate lower than 70 ml/min *Proteinuria or bacteriuria did not* occu in any case All subjects had previously been investigated with respect to the trace element concentration in serum and rine before treatment (19).

Blood samples, taken in fasting state were drawn through plastic catheters inserted into cubital vein and the first 10 ml of blood was discarded After centrifugation 1 ml samples of serum were pipetted into quartz ampoules, which were rapidly flame-sealed as described previously (20) On the same day urine was collected for 24 hours in thoroughly cleaned polythene bottles. Samples of 1–2 ml of rine were pipetted into quartz ampoules, which after flame-sealing were ready for irradiation The quartz ampoules were irradiated together with standards in the R 2 reactor at Studsvik at thermal neutron flux of $2 \cdot 10^{12}$ $n/cm^2/$ sec for 24 hours The chemical separation procedure and the γ-spectrometric measurements and identification of the elements have been described elsewhere (1 12 18).

Statistical methods

In the statistical analysis Student -test has been used in comparing serum concentrations and the paired test in comparing urinary excretions. The degrees of significance obtained have been expressed as. – not significant ($p>0.05$) almost significant ($0.01<p<0.05$) significant ($0.001<p<0.01$), ** highly ($p<0.001$).

Table I *24-hour urinary excretion of trace elements with known biological function before and after 6 months treatment with chlorthalidone (μg/24 h)*

	Co		C		Fe		Zn	
Case no.	Before	After	Before	After	Before	After	Before	After
1	0.44	0 6_	28	46	51	70	310	440
	1.8	0 74	37	45	130	56	350	410
3	1 7	0.94	37	76	160	180	460	570
4	0 45	0 77	37	38	59	140	430	1 020
5	0 67	1.8	4	35		170	140	660
6	0 59	1.6	77	49	67	110	800	1 035
Mean±S D	0 94±0 63	1 1±0 50	37±24	48±15	93±49	121±51	420±220	690±280
Mean difference	+0_4		+1		+19		+274	

RESULTS

The results of trace element analysis of the 24-hour urinary samples collected after 6 months treatment with chlorthalidone are given in Tables I–III In these Tables as well as in Tables IV–VI the trace elements have been divided into those with known (Table I) suspected (Table II), and without known biological function (Table III).

Among the trace elements with known biologi cal function zinc was present in an almost significantly higher amount in the urine after 6 months treatment than before treatment and among the trace elements with suspected biological function the rubidium excretion was almost significantly higher after treatment The urinary excretion of bromine cadmium and molybdenum after 6

Table II *24-hour urinary xcret on of trace elements with suspected biological function before and after 6 months treatment th chlorthalidone (μg/24 h)*

	Br		Cd		Mo		Rb	
Case no	Before	After	Before	After	Before	After	Before	After
1	900	3 300	1 9	1.5	33	78	1 600	2 900
2	100	3 900	1 8	2 4	89	250	000	200
	5 500	9 800	0 78	3.2	87	200	600	800
	3 700	8 900	0 70	0 70	87	150	3 200	5 900
	1 700	5 700	0.54	4.3	34	90	2 000	4 000
	3 700	6 300	1 0	2 7	95	95	3 300	3 100
±S D	3 700 1 400	6 300± 700	1 1±0 99	5±1.3	71±29	170_73	900±700	3 400 1 200
Mean difference	3 090		+1 4		99		+900	

Table III *24-hour urinary excretion of trace elements without known biological function before and after 6 months treatm nt th chlorthalidone (μg/24 h)*

	Ag		As		Au		C	
Case no	Before	After	Before	After	Before	After	Before	After
1	0 32		230	17	0 00058	0 0071	8.8	12
2	1 1	0 33	120	98	0 0058	0 00070	8 1	15
3	0 43	0 5	370	93	0.0010	0 00056	14	8 7
4	0 44	0	570	260	0.083	0 0017	17	26
5	0.87	0 76	58	77	0 0020	0 0098	13	_5
6	0 40		110	1 300	0 00052	0.00088	14	4
Mean±S.D	0.60±0.31	0 46 0 4	40±200	310±300	0 016_0 033	0.0035±0 0030	13±3	19±7
Mean difference	−0.20		+43		−0 01		+5 4	

months treatment was also high but not significantly higher than before treatment. None of the trace elements without known biological function showed significantly increased or decreased excretion after 6 months treatment.

The mean values and standard deviations of the trace element concentrations in serum before and after 6 months treatment are given in Tables IV–VI. None of the 17 trace elements investigated, with the exception of rubidium showed any significant difference in serum concentration before and after 6 months treatment. The values for cadmium caesium cobalt gold mercury and scandium were low after treatment while copper and molybdenum values were high. These differences were however not significant.

DISCUSSION

In a previous study of trace elements in serum and urine from hypertensive patients before and after 5 days treatment with chlorthalidone (19) highly significantly increased amounts of bromine rubidium and zinc and almost significantly increased amounts of cobalt and cadmium were observed during treatment. However the serum concentration of only rubidium was significantly lower after 5 days treatment. If the increased urinary excretion of some trace elements found during short-term treatment were to continue during treatment for longer periods one would expect that deficiency might develop. In the present study after 6 months treatment high urinary excretion and low serum concentrations were observed for some of the elements investigated. In the following discussion the elements will be dealt with in the groups used earlier i.e. trace elements with known suspected and without known biological function.

Trace elements with known biological function

Cobalt The serum concentration was lower and the urinary excretion higher after 6 months treatment than before treatment but the differences were not significant. An almost significant increase in urinary excretion during 5 days treatment has previously been observed (19) and it is possible that too few cases were investigated in the present study to obtain significant differences.

Copper The mean serum concentration of copper after 6 months treatment was high (1.07 μg/ml) as compared to before treatment (0.80 μg/ml) and to the concentration after 5 days treatment (0.84 μg/ml). The difference between 1.07 and 0.80 was however not significant. The mean value after 6 months treatment almost the same as the mean value previously found in serum from 8 healthy individuals. The urinary excretion of copper after 6 months treatment was not significantly changed.

Se	
Befor	After
[illegible]	3
[illegible]	40
33	
43	44
46	31
44	54
32 ±11	38±1 6.4

Hg		Sb		Sc		W	
Before	After	Before	After	Before	After	Before	After
0.98	0.4	9	0.71	0.090	0.0075	9.7	1
1.0	0.74	1.8	1.6	0.011	0.0088	6	15
1.18	0.79	1.4	1.3	0.035	0.0069	3	1
99	8	1	1.3	0.0049	0.004	44	41
1.16		1	1.3	0.36	0.076	1	1
0	0.91	1	1.6	0.0039	0.0051	13	1.8
65±0.41	1.0 0.58	1.5+0.3	1.6+0.84 0.77	0.083±0.14	0.019+0.028 0.061	13 1	70 14

Table IV *Serum concentration of trace elements with known biological function before and after treatment with chlorthalidone (mean±S.D μg/ml)*

	Untreated	After 6 months treatment
C	0 00044±0 00015 (*n*=5)	0 00036±0 00016 (*n*=5)
Cu	0.80±0.31	1 07±0 27
Fe	1.26±0 53	1.37±0 74
Zn	0 91±0 11	0.83±0 12

Iron No marked changes in the iron concentration in serum or urine in relation to treatment with chlorthalidone were observed

Zinc The amount of zinc in urine after 6 months treatment was almost significantly higher than before treatment The mean value of 690 μg/24 h was almost as high as that (720 μg/24 h) found after 5 days treatment which was highly significantly different from the mean value of 520 μg/24 h before treatment. Thus the previously observed increase in zinc excretion after short term treatment persists after long-term treatment. However the serum concentration after 6 months treatment was not significantly lower than before treatment Enhancement of renal excretion of zinc by hydrochlorothiazide has recently been observed by Pak et al (6) They found that the increased urinary zinc excretion also persisted after 1 month s treatment and that zinc concentration in serum was unchanged or within the upper range of normal after treatment for 1 year or more

T ace elen t with su pected biological function

mine The urinary excretion of bromine was , but n t significantly higher after 6 months tment tha before treatment If the mean value for urinary bromine excretion in 15 untreated hypertensive patients previously investigated is compared to the mean value for the urinary excretion of bromine from the 6 patients after 6 months treatment an almost significant increase in excretion is found for the latter group. However the concentration of bromine in serum was almost the same after 6 months treatment as before.

Table V *S rum nc nt ation of trace element with suspected bi logic al func t on before and after treatment with hl rthalidone (mean±S.D μg/ml)*

	Untrea d	After 6 months treatment
Br	3.8±1.5	3 8±1 7
Cd	0 0018±0 0018	0 0013±0 0014
Mo	0 0049±0 0054	0 010±0 005 (*t*=2 15)
Rb	0.24±0.03	0 17±0 02 (*t*=4 03)
Se	0 17_0 05 (=4)	0 18±0.07 (*n*=4)

Table VI *Serum concentration of trace elements without known biological function before and after treatment with chlorthalidone (mean±S D μg/ml)*

	Untreated	After 6 months treatment
Ag	0 61±0 18	0 55±0.24
As	5 9±5 1	6 7±9 4
Au	0 0041±0 0036	0 0017±0 0028
Cs	1.5±0 95	1 1±0.58
Hg	2.3±1 4	1 7±1.2 (*n*=5)
Sb	0 71±0 77	0 77±0.36
Sc	0 15±0.21	0 011±0.013
W	5.2±5.3	11±14

Cadmium The urinary excretion of cadmium after 6 months treatment was high The paired mean difference between the excretion before and after 6 months treatment was not significant but the *t* value was 2.0 Calculation with Student s *t*-test showed an almost significant difference. After 5 days treatment an almost significant rise in urinary excretion was previously observed The serum concentration after 6 months treatment was low but not significantly lower than before treatment.

Molybdenum A high mean value of molybdenum was noted in serum after 6 months treatment, but as compared to the mean value before treatment there was no significant difference The urinary excretion after 6 months treatment did not differ significantly from that before treatment when calculated on paired mean differences Calculation with Student s *t* test however showed a significantly higher excretion after treatment.

Rubidium After short-term treatment with chlorathalidone a highly significant increase in the urinary excretion and an almost significant decrease in the serum concentration of rubidium as compared to before treatment have previously been found (19) After 6 months treatment the urinary excretion was also high and differed almost significantly from that before treatment, and

the serum concentration was highly significantly decreased as compared to before treatment Rubidium usually behaves in the same way as potassium. However all the patients treated for a long time with chlorthalidone received potassium chloride in addition and no hypokalaemia was noted

Selenium No marked changes in serum concentration or urinary excretion of selenium in relation to treatment with chlorthalidone were noted

Trace elements without known biological function

The variability in serum concentration and urinary excretion of the trace elements without known biological function was large In the previous study the serum concentration and urinary excretion after short-term treatment with chlorthalidone was compared to that before treatment a significant difference was only obtained for caesium which showed a decreased urinary excretion after treatment. When the values after long-term treatment with chlorthalidone were compared to those before treatment, no significant differences were obtained for any of these elements

In conclusion the previously observed increase in urinary excretion of bromine cadmium cobalt rubidium and zinc after short-term treatment with chlorthalidone was also present after long-term treatment with regard to rubidium and zinc and possibly also to bromine cadmium and cobalt However a significantly lowered concentration in serum after long-term treatment was only found for rubidium

Whether a lowered concentration of rubidium in the serum has any pathological significance in human beings remains uncertain That the action of rubidium on the contraction of the isolated frog s heart was almost identical with that of potassium was observed already by Ringer (11) Furthermore the same influence has bee found from the extracellular ionic concentration of potassium nd rubidi m on the resting potential nerve and muscle preparations and on the configuration of the ECG (10) Thus it may be possible that a lack of rubidium may ha similar effects to lack of potassium or at least potentiate the effect of n already developed hypokalaemia

The incre sed urinary xcretion of zinc was not accompanied by a ignificantly decreased concentration n serum which may be explained either by increased gastrointestinal absorption by mobilization from tissues If a tissue zinc deficiency develops during long-term treatment with chlorthalidone and hydrochlorothiazide it may be of clinical significance Zinc is known to be an essential trace element It constitutes a part of the molecule of several enzymes connected with carbohydrate and protein metabol sm. Zinc deficiency is known to cause various pathological conditions in different animals (17) In man however the picture is not as clear Dwarfism (9 14) and impaired tissue repair (8 13) have been reported as well as a connection with beri-beri, porphyria, postalcoholic cirrhosis and diabetes mellitus (2, 3 4) Recently a rare skin disease acrodermatitis enteropatica, has been reported to be cured by oral administration of zinc (5) The role of zinc in these various diseases is however not fully understood

ACKNOWLEDGEMENTS

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ZINC PROTAMINE-GLUCAGON IN THE TREATMENT OF PAGET'S DISEASE OF BONE

PRELIMINARY REPORT

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Paget s disease of the bone is rarely painful How ever the treatment of these rare cases has until recent years been poor

A symptomatic and biochemical effect has been shown by treatment with mitramycin (4 7) and actinomycin (5) but the toxicity of these drugs makes them unsuitable for treatment of ambul tory patients. *Calcitonin has been reported to nduce* clinical and biochemical remission (2 8 9) but the half-life of the hormone is so short that at least or 3 daily injections are requ red A most striking and dramatic effect on the clinical and biochemical f nd ings has been demonstrated during treatment with i v infusion of glucagon (3)

Due to the difficult administration of both calcito nin and glucagon and the effect of the latter we here report the biochemical and clinical results from a patient with Paget s disease of bone treated w th zinc-protamine-glucagon w th prolonged action

METHODS

Total urinary hydro yproline was measured by the methods of Bergman and Loxley (1) Serum alkaline phosphatase was measured by the method described by March et al (6) calcium in serum and urine by flame spectrophotometry

CASE REPORT

A 70-year old man with intolerable pai in the femur and radiological and bioptical evidence of Paget disease of bone was studied

Initially serum calcium was 105 mg/l, plasma phosphate 28 mg/l and serum alkaline phosphatase 1 6 King Armstrong units/100 ml

The patient treated with zinc-protamine glucagon (suggested and supplied by Nov Industry A/S) mg/day subcutaneously one daily injection f r 14 da and thereafter 5 mg/day for the next 3 weeks The treatment resulted in fall in rinary calcium total urinary hy dro yproline and serum alkaline phosphatase (F g 1) Serum calcium nd plasma phosphatase were nearly un changed during the therapy

A dramatic effect the bone pain occurred after initia tion of the treatment The night after the first injection the patient suffered no bone pain at all and he slept undisturbed for the first time in three month

Rather pronounced side-effect occurred during the first series of treatment ith 5 mg zinc-protamine-glucagon His resting pulse rose from 60 to 80 beats/min his BP rose from 120/80 to 170/90 mmHg and he experienced mental imbal ance These side-effect disappeared after interruption of the treatment

Half year after the first series he had relapse of his pain in the left femur H treated with 1 25 mg zinc protamine-glucagon for 8 days Again his ymptoms disap peared immediately and on this dose he had no side-effect

DISCUSSION

Normalizing of the clinical and biochemical signs of Paget s disease of bone treated w th inc protamine-glucagon has not previously been e ported.

I ou patient the treatment produced a reduction of serum alkaline phosphatase total urinary hydro yproline nd 4-hour urinary calcium Furthermore the patient experienced complete relief of bone pain The results confirm the findings of Condon (3) who treated 4 patients w th glucagon given by i. infusion

Using zinc-protamine-glucagon only one dail

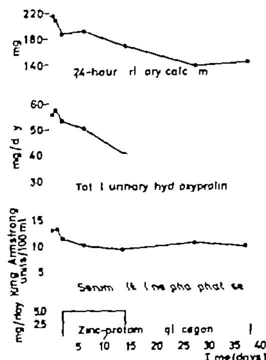


Fig 1 Level of 24-hour urinary calcium, total urinary hydroxyproline and serum alkaline phosphatase during treatment with zinc-protamine-glucagon.

jection is required and that facilitates the administration of the therapy The side-effects are considered to be negligible especially when only 1 mg/day is administered

The presented results suggest that zinc protamine-glucagon may be useful in the treatment of Paget s disease of bone but further trials are clearly necessary

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BONE MINERAL IN SARCOIDOSIS

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Abstract The uptake of ⁸⁵Sr in the spine and both the bone mineral linear density (g/cm) and mineral density (g/cm²) of the trabecular radius, midshaft radi s and ulnae have been measured in 21 untreated sarcoid patients using the ²⁴¹Am γ-transmission method The results were compared with control subjects who showed no evidence of bone involvement or disturbances in calcium kinetics. Sarcoidosis wa considered to be subacute active in 14 cases (5 male and 9 female), subacute inactive in four (1 male and 3 female) and chronic active in three (1 male and 2 female). The study showed. (*a*) In women the mean ⁸⁵Sr uptake in the spine was significantly smaller than in the controls. (*b*) I the early stages of the disease the ⁸⁵Sr uptake values were lower than when the disease was prolonged both in men and women. (*c*) The bone mineral linear den ty of the trabecular radius in active subacute sarcoidosis was significantly higher than in the controls. The relative difference in mineral density was of the same magnitude (*d*) In chronic sarcoidosis neither the ⁸⁵Sr uptake in the spine nor the bone mineral values differed from the control values. (*e*) The mineral linear density and density in the midshaft radius and lnae were at the same level as in the controls. (*f*) A significant negative correlation was established between the ⁸⁵Sr uptake in the spine and the bone mineral density in the distal radius It seems evident from this study that patients in the acute phase of sarcoidosis have an abnormally high bone mineral density and that there is distinct mineral decrease correlated with the duration of the disease

Changes in calcium metabolism are known to occur in conjunction with sarcoidosis The incidence of hypercalcemia has ranged from 0 7 to 6..5% in different studies (18 28). With the ex ception of the materials eported by Rudberg-Roos (31) and Sharma et al (35) hypercalcemia has been rare in Scandinavia and Great Britain In Finland lev ted serum calcium values were established by Putkonen et al (30) in only 3.3% of 60 patients with sarcoidosis Hypercalciuria has been found to be 2-3 times more common than hypercalcemia (38) Changes in calcium metabolism are evidently more common in chronic and widespread forms of the disease (11) Since Schaumann (33) published his studies it was be lieved that disturbances of calcium metabolism are caused by the bone manifestations of sarcoidosis until it was observed that there was no correlation between the changes in question (14 27) Demineralization of bone secondary to sarcoidosis has been observed rarely (22) However the sensitivity of X-ray studies in the evaluation of bone density is known to be poor since the mineral loss must be at least 30% before it can be demonstrated (15)

Study of the uptake of ⁸⁵Sr has recently gained an established role i the evaluation of bone secre tion (6, 9) On the other hand the γ-transmi ssion method has proved to be a highly sensitive and reliable proced re for study ng bone mineral content in vivo (2, 36) However we have been unable to trace a study n which bone density has bee measured by this method in patients with sarcoidosis

The purpose of our work was to study bone mineral turnover n patients with sarcoidosis by measuring the uptake of ⁸⁵Sr in the spine and the bone mineral content using the Am γ-ray transmission method

MATERIAL

Twenty-one untreated sarcoid patients—14 omen and 7 men—were studied The female age range was 33–7? years mean 48 and the male 31–74 years mean 48 The patients had not taken hormones, vitamin D or other drugs know to affect the calcium metabolism Other diseases which might be of significance for calcium kinetics and bone mineral balance ere ruled out by clinical and laboratory tests. None of the patients

Table I ^{85}Sr uptake in the spine of controls and patients with sarcoidosis

^{85}S uptake (counts/? ml)

Controls		Patients		Significance*	
	Mean±1 S D		Mean±1 S D	*t*	*p*
Females					
13	3 590±870	14	2 820±960	_18	<0 05
Male					
22	2 980_840	7	2 650±390	1 42	>0.05

Student *t* test was used for the statistical analysis.

displayed clinical or roentgenological evidence of hyper trophic pulmonary osteoarthropathy

The diagnosis of sarcoidosis was based on a typical disease picture and biopsy except in two patients for whom histological verification was missing but who were found to have characteristic hilar gland enlargement, one had negative Mantoux ad 100 TU and the other iridocyclitis. Enlarged hilar glands were encountered in 20 cases, lung parenchymal changes in 8 Four patients had characteristic skin changes and one had iridocyclitis The onset of the disease was estimated in 14 cases from the subjective ymptoms and in 7 on the basis of the changes seen at mass miniature X-ray The exact time of onset is naturally uncertain especially in the last mentioned group.

The patients were divided into three groups according to the disease picture Group I patients with subacute active sarcoidosis of les than two years duration (14 cases 5 men and 9 women) group II patients with subacute inactiv sarcoidoses which had lasted two years at the most and who were subjectively asymptomatic and displayed only minimal roentgenographic residual (4 cases, 1 man and 3 women) group III patients with chronic sarcoidosis (3 cases 1 man and women) whose disease had lasted for three four and ten years and in hom the diagnosis had been fied clinically and histologically The control group the ^{85}Sr measurement comprised 29 subjects (19 and 10 women) The age of the men ranged from to 61 years, mean 45 and that of the women from 32 to 87 years mean 47 The measurements were made during the same period as for the patients The indication for radiostrontium examination these cases was low back pain, there was no X-ray or clinical evidence of local or generalized disease affecting the bone or calcium kinetics. The control group for th mineral measurements has been presented earlie 1)

METHODS

The ^{85}Sr uptake in the spine of the subjects was measured using the standard method for our laboratory ((I. The tracer used 50 μCi of ^{85}Sr as strontium chloride was administered i one week before the count. The counting apparatus consisted of a 1 75×?" NaI(Tl) scintillation detector and an analyzer-scaler device The radioactivity in bone was counted from the spine Measurements were made in a skin field of 3×5.5 cm. The crystal-ski distance was 10 cm. Background measurements were performed by covering the measuring field with a 1 7 cm thick lead shield. The measuring time was min. The mean of five measurements in the Th VIII–XII region was entered as the uncorrected result. The result was corrected for the injected dose by measuring the activity of the tracer syringe before and after injection by a standard measurement geometry and comparing it with the activity of a standard dose selected by us. The stability of the measuring equipment was taken into consideration by measuring with standard geometry a certain ^{85}Sr standard while giving the injection and while taking the count, and taking into account the 65-day half-life of the ^{85}Sr radionuclide measured.

The bone mineral was measured by a modification of the γ-radiation attenuation method (20) The source of the radiation was 45 mCi ^{241}Am radionuclide γ-energy 60 k V The forearm to be measured was fixed in a waterbath between the source and detector At the measuring site of the bone the result recorded was the mean of four scans across the bone The measurement was mad about 1.5 cm from the distal end of the right radius i the region of cancellous bone The mineral density (g/cm) was calculated from the equation

$$\varrho = \frac{K\lambda_1}{A} \quad \text{where}$$

$$K = 1 + a \exp\left(-b \sum_{=1} \ln \frac{I}{I} /A\right)$$

$$\lambda = \frac{\Delta r \varrho_m}{\mu_m \varrho_m - \mu_s \varrho_s} \sum_{=1} \ln \frac{I}{I}$$

or the mineral linear density (g/cm) of the bone and $A(0\ 47(d^2+1))$ is the cross-sectional area of the bone at the measuring site The other ymbols are: $a = 5\ 4?$ $b = 4\ 95\ cm^2$ I = photon fluence rate that has penetrated through water (soft tissue) I = photon fluence rate that has penetrated bone and ater μ_m = mas attenuation coefficient for bone mineral (0 395 cm²/g), ϱ_m = microscopic density of bone mineral (2 70 g/cm³) μ_s = mas attenuation coefficient for water (soft tissue) (0 205 cm²/g), ϱ_s = density of w ter (soft tissue) (1 00 g/cm³) Δr = the distance of the points acros the bone where the summation of l (I_o/I) wa ade (0 10 cm) n = the numbe of the points from which th sum is calculated

On the assumption that the shape of the cross-section of the compact bones in the forearms is circular mineral density index was calculated from $\varrho = \lambda/A$ where $A = \frac{\pi}{4} (d^2 - d'^2)$ d and d' are the outer and inner diameters of the bone measured from X-rays. Measurement *of cortical bone were made at the point between the* middle and distal thirds of the radius and ulna. The

Table II *Mineral linear density and mine al density of the distal radius of the patient and controls*

Density	Group		Bone mineral Patients (mean±1 S.D.)	Controls (mean±1 S.D.)	Difference (%)	Significance* t	p
λ (g/cm)	I	14	1.43+0.28	1.30+0.29	10.0	3.47	<0.005
	II	4	1.34 0.25	1.27+0.27	5.5	0.5?	>0.05
	III	3	1.12+0.30	1.18+0.28	−5.1	−1.00	>0.05
	♀+♂	1	1.37+0.29	1.28+0.27	7.0	.49	<0.025
	♀	14	1.[illegible]1+0.18	1.10 0.08	10.0	[illegible].76	<0.02
	♂	7	1.[illegible]4+0.08	1.64+0.1[illegible]	0.0	0.00	>0.05
ρ (g/cm³)	I	14	0.310+0.06[illegible]	0.282+0.028	9.9	1.95	>0.05
	II	4	0.304+0.037	0.288+0.015	5.6	0.6[illegible]	>0.05
	III	3	0.257 0.043	0.258+0.007	−0.4	−0.03	>0.05
	♀ ♂	21	0.301+0.057	0.280 0.025	7.5	1.9[illegible]	>0.05
	♀	14	0.291+0.047	0.270+0.016	7.8	1.83	>0.05
	♂	7	0.321+0.074	0.300+0.030	7.0	0.83	>0.05

Student t-test and paired comparison were used for the statistical analyses.

methods used and the constants required ha e been presented in detail earlier (1).

I the statistical analysis of the results of bone mineral measurements comparison was made with normal material (1). The control values were taken at point corresponding to the patient s age from the regression line for the right hand The regression line was calculated in the range of 30–81 years for women and 30–87 years for men.

X-ray examination of hands and the lumbar spine was performed in all the patients and serum alkaline phosphatase and creatinine were also measured in all Serum lectrophoresis was performed in 19 patients A total of 66 serum calcium and 63 serum inorganic phosphorus determinations were made by testing all but tw of the patients at least twice. The test was performed only onc in these tw cases. During standardized diet containing 800 mg calcium and 1 200 mg phosphorus the 4-hour urinary excretion of calcium as measured in 20 patients and phosphorus excretion in 19 The 4-hour urinary hydro yproline excretion was studied in 18 cases while on gelatin-free diet.

RESULTS

The results for ⁸⁵Sr uptake i the spine are given in Table I The bone mineral linear density and mineral density of the distal radius are presented in Table II The difference $\Delta\rho$ $(\rho_{pat} - \rho_{norm})$ in the mineral density was negatively correlated with ⁸⁵Sr uptake The correlation was statistically significant for the total material (= 1 = −0.51) and for women (=14 = −0.60) For men too there was a tendency but it was tatistically non-significant (=7 r=−0.53) In group I the $\Delta\rho$ w negatively related to the mea ure ments for the duration of the disease. The correlation was not statistically significant (n=14 =0.29) (Fig. 1). In the diaphysis of the radius and ulna there was no statistically significant difference in the linear density nd density between the controls and the patients. The per cent difference was mostly smaller than 5

Only minor degenerative changes and positional errors in the lumbar spine were demonstrated on X-rays. Table III shows a part of the laboratory results

DISCUSSION

Changes in the calcium metabolism in sarcoidosis established n earlier studies have been similar to those seen in vitamin D intoxication and even small doses of vitamin D have been toxic for some patients with sarcoidosis (8 17 [illegible]5 3[illegible]) It has therefore been thought that it might be a question of an endogenous sensitivity not accurately

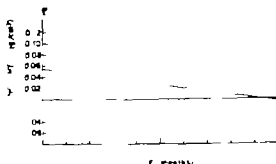


Fig. 1 Correlation between the mineral increment and the duration of the disease to the measurement in group I

Table III *Clinical and laboratory data of 21 sarcoid patients*

Pat. no	Sex	Age (y)	Group	Duration from verification of disease to examination (mo)	Mean serum calcium (mmol/l)	Mean serum phosphat (mmol/l)	Serum alkaline phosphatase (U/l)	Urinary calcium excretion (mmol/24 h)	Urinary phosphate excretion (mmol/24 h)	Tubular phosphate reabsorption (%)	Urinary hydroxyprolin excretion (µmol/m²/24 h)	Serum creatinine (µmol/l)
1	♀	42	I	3	2.40	0 83	3	4.88	26 3		170	95
2	♀	52	I	9	2.85	1 05	27	8 90	29.2	79		60
3	♀	38	I	9	2.45	1 11	20	6 95	39.8	75	130	60
4	♂	64	I	5	_50	1 17	17	9.36	43 1	72	137	95
5	♂	31	I	2	2.50	1 08	19	6.40	36.5	85	157	100
6	♀	54	I	4	2 40	1.35	49	2.84	36.4	71	117	75
7	♀	46	I	2	_50	1 00	20	5 74	26 0	89	104	85
8	♂	33	I	1	2.50	1.30	48	5.35	79 4	89	168	95
9	♂	4	I	5	2 55	1.25	23	4 15	70 9	95	116	85
10	♂	42	II	5	2.40	0 98	34	2.33	31.8	72	115	90
11	♂	74	III	120	2.40	1.20	79				145	130
12	♀	33	I	4	_35	0 96	28	5 04	50 5			95
13	♂	46	I	4	2.25	0.83	27	7 60	45 4			75
14	♀	72	I	9	2 5	1 20	32	2.60	10.2	90	103	75
15	♀	60	III	36	_50	1 18	44	2.88	6.1	96	76	80
16	♀	50	II	1	2.40	1 04	25	3 34	6.8	94	97	90
17	♀	59	I	3	40	1 1	41	8.25			95	95
18	♀	41	II	5	2.45	1 15	13	4.50	79 0	73	113	95
19	♀	53	III	48	40	1 04	20	2 48	22 1	82	97	75
20	♀	34	I	7	_35	1 00	19	3 48	28 3	82	115	55
21	♀	35	II	12	2 40	1.33	21	3 12	27.8	87	84	65
Normal					2 25–2 75	0 81 1 45	13–49	1.3–7.3	<26 0	70–90	63–179	60–115

known to vitamin D (3 5 13 27 38) On the other hand the sensitivity to vitamin D of different organs (gastrointestinal tract bones kidneys) varies from one individual to another (10 17)

The results of the calcium balance studies performed have varied with the test arrangements e g. the magnitude of the calcium intake (3 8 14 16 17 26 27) The disease of practically all the patients has been chronic and accompanied by hypercalcemia and/or hypercalciuria and renal insufficiency some of the patients have received vitamin D These circumstances make interpretation of the results difficult and generalization impossible The calcium kinetics of bone has been studied only in a few selected patients The calcium turnover of bone has been either slower (23) faster (7 16) than normal Chronic overdosage vitamin D has led to bone mineral loss (37) sarcoidosis could also be expected to lead to increasing porosity of bone In spite of the great interest in the changes that occur in the calcium metabolism no studies on the bone density of sarcoid patients have been published so far

Anttonen et al (4) reported higher than normal spinal ⁸⁵Sr uptake values in patients with sarcoidosis whose disease was chronic In our work the uptake of ⁸⁵Sr was lower in sarcoidosis than in a normal material which suggests that the rate of bone accretion was slower than normal at the time of examination The difference was significant for the women The men displayed a similar tendency However the ⁸⁵Sr uptake values for both women and men were higher in the later phase of the disease than in the patients who were examined in an early phase The difference was not statistically significant however

Bone mineral linear density was distinctly (approx 10%) higher in subacute active sarcoidosis than in the controls The difference was statistically significant In this group the difference in mineral density (g/cm^3) too was always of the same magnitude but because of greater deviation was not statistically significant Bone density was higher in the measurements made in the early phase of the disease than in the cases with longer duration of the disease

The results suggest that the increase in bone

mineral density occurs before the clinical manifestation of the disease since the tendency to slow decrease in bone density was demonstrable already in the first months after diagnosis of the disease

A statistically significant negative correlation was established between the uptake of ⁸⁵Sr and the density of cancellous bone

The results for patients with subacute inactive sarcoidosis were similar but less marked.

Bone density was smaller in two out of three patients with chronic sarcoidosis than in the controls. The result is not in disagreement with earlier information. Individual cases with increased radiolucency of bone have been reported but their disease was chronic and on account of hypercalcuria led to renal insufficiency (22) In our series one patient with chronic sarcoidosis had a slightly elevated serum creatinine value On the other hand Jackson and Dancaster (17) and Henneman et al. (14) drew attention to the rare occurrence of bone demineralization in spite of sarcoidosis lasting for years As cortisone and its derivatives have been n common use for a long time now it is difficult to get reliable information on bone changes caused by the primary disease

In contrast our observation that mineral density is higher than normal n cancellous bone in the initial phase of the disease is not mentioned in the literature It seems that evaluation of bone density in sarcoidosis is extremely difficult as the results depend on establishing the duration of the disease which often impossible It is often not possible to estimate the onset of the disease in practice with sufficient accuracy because of the paucity of symptoms and the great number of asymptomatic cases that are detected at X-ray examination—39% in F nnish study (34)

Mineral linea density and mineral dens ty meas ured from the midshaft radius nd ulna did not differ gnificantly from the control mea urements This is explained by the considerably f ter rate of mineral changes n cancellous tha cortical bone (19)

Bone X-ray revealed nothing abnormal apart from minor osteochondrous and pos tional err rs of the lumbar pine Serum electrophoresi howed slight elevation of α- and/or β-globulin 10 patient The album n fraction w elevated 55 g/l n only one of the patient (normal 33–44 g/l). In addition a slight decrease in γ-globulins was observed in one patient with subacute and a slight increase in one with chronic sarcoidosis.

One patient in group I had hypercalcemia and hypercalciuria and another three had hypercalcuria as well. Their uptake of ⁸⁵Sr was of the same magnitude as in the other patients of the same group The hypercalciuria and hyperphosphaturia established in 13 patients may have been caused by the fast absorption of these minerals from the intestines.

Urinary excretion of hydroxyproline has been observed to correlate well with the bone resorption rate (23 29) The 4-hour urinary hydroxyproline excretion in our tudy was within the normal range The increase i bone resorption in prolonged sarcoidosis is evidently such a slow process that it is not detectable by this kind of investigation

Elevation of alkaline phosphatase of bony origin the serum is correlated with accelerated mineral accretion (_3). No elevated values of th enzyme were observed by us which accord well with the ⁸⁵Sr uptake tudies

It has been shown in recent years that the effect of vitamin D on active absorption of calcium from the intestine is due to the activation of enzymes the synthesis of a specific transport protein and the accumulation of phospholipid the membrane of the microvilli of mucosal cell (5 1). To the best of our knowledge the connection between the abundant phospholipid formation that occurs in sarcoidosis granulomas (30) and the metabolism of intestinal phospholipids ha not been studied

A theoretical hypotheses that might be considered is that the increase in phospholipid in the intestinal mucosal cell of sarcoid patient in the phase of origin of the disease tend to lead to accelerated absorption of calcium and phosphorus The supply of mineral t the bones then ncreased which turn lead through so far unknow mechanism to the growth of bone mineral density The possibilit f latent pulmonary osteopathy cannot be ruled out Although th osteopathy ha not been described connection w th sarcoidosi Y ncey et al (39) rece tly observed the clubbing phenomeno 1 4 of 136 patient w th sarcoid si On the other hand. lubbing may be the first and only ymptom of

pulmonary hypertrophic osteopathy (4) A condition reminiscent of vitamin D intoxication which may be encountered in chronic sarcoidosis seems to cause gradual demineralization of bone Elucidation of these points calls for further studies of *ufficiently large unselected and untreated materials*

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LETTER TO THE EDITOR

WEIGHT AND IODINE CONTENT OF THE THYROID GLAND IN JUTLAND DENMARK

Sir

No report concerning the size of the thyroid gland in Denmark has ever been published, and no figures for iodine concentration of the gland in this area are available. Small glands are known to be iodine-rich (4) and goitres to be iodine-poor. An association between iodine concentration and size of goitres has been found (1, 2) but no statistically significant correlation has been shown.

Thyroid glands from 61 females and 156 males aged 15–90 years brought to medico-legal autopsy collected by the staff of the Institute of Forensic Medicine Århus were investigated. These persons came from all parts of Jutland and most of them were healthy before the accident causing death. All fat and *muscular tissue was removed before the glands were* weighed and thereafter homogenized in water to a tissue concentration of about 40 mg/ml. Duplicate estimation of iodine concentration of the homogenate was done in the Clinical Laboratory Århus Kommunehospital with an auto-analyser technique. Based on the first 100 duplicate estimations the S D was 5%.

Distribution curves for thyroid weight are given in Fig. 1. The arithmetic mean weight of the female gland was 22.9 g (median 19.3) and of the male 25.5 g (median 22.8). The weight of the gland did not vary with age but was negatively correlated to iodine concentration of the tissue both in females ($p<0.01$, $r=-0.31$) and males ($p<0.0025$, $r=-0.24$). A positive correlation between body weight and thyroid weight was found for males ($p<0.0005$, $r=0.35$).

Iodine concentration was 374 μg/g wet thyroid tissue ± 182 for females and 4 4 μg/g ± 177 (mean $\pm$S D) for males.

In West Jutland thyroid weight was lower (mean 15.8 g against 24.8 g, $p<0.01$) by Wilcoxon's rank sum test and iodine concentration higher (mean 496 μg/g against 322, $p<0.0025$) than in East and North Jutland, statistically significant only for females. The same tendency was found for male glands. This difference between west and east+north could be due to a washing out of iodine from the soil in east+north during the last glacial period (3), or because iodine-rich rain comes from the west (5) resulting in larger iodine intake through food for the people in west Jutland.

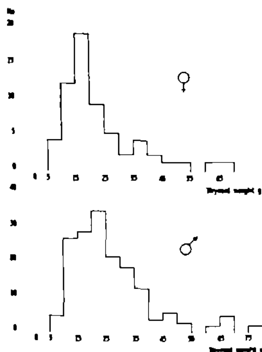


Fig. 1. Thyroid weight in males and females.

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THE FUNCTIONAL PATTERN OF THE TRANSPLANTED LIVING-DONOR KIDNEY DURING THE EARLY POSTTRANSPLANT PERIOD

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Abstract. Renal function has been studied within the first 20 days after renal transplantation in 14 recipients who had received kidney from a closely related living donor. There was a good correlation between blood flow measured with an electromagnetic flowmeter and ERBF calculated on the basis of PAH and ¹³¹I-hippuran clearances. The ERPF reached 70% of the donors' bilateral preoperative ERPF during the course of –3 hours. However the recipients' ERBF averaged only 70% of the blood flow measured in the renal artery. During the course of 20 days the recipients' glomerular filtration rate increased to 67% of the donors' bilateral preoperative value. Immediately after transplantation a change in function took place, significantly reduced filtration fraction being seen. During the course of the first 20 posttransplant days the filtration fraction approached the normal range.

Renal grafting involves implantation of a denervated organ which has been ischemic. This discontinuance of nervous regulation of renal circulation might, together with an increased sensitivity to circulating vasoconstrictive substances, affect the glomerulotubular balance (8). Mean effective renal blood flow (ERBF) has previously been studied after transplantation using [13] xenon (1, 7), electromagnetic flowmeters (1, 5), para-aminohippuric acid (PAH) and ¹³¹I-hippuran clearances (1, 6, 10). Immediately after transplantation the relationship between mean ERBF and glomerular filtration rate (GFR) changes, resulting in a fall in the filtration fraction (FF) (1, 6). This change in hemodynamics is revealed by the isotope renogram as a change in relationship between speed of uptake and elimination and relative reduction in the speed of elimination (3).

The purpose of the present study was to investigate functional patterns of the transplanted kidney during the first three weeks after transplantation. These functional patterns were compared with functional patterns of living renal donors immediately before nephrectomy.

MATERIAL

Fourteen donors, seven men and seven women, aged 19–60, were studied immediately before renal allotransplantation (RAT). All had normal renal function (11) (Table I).

Of the 14 recipients, aged 14–53, receiving kidney from these donors eight were men and six women. Each patient was studied three times, immediately after the transplantation, –6 days later and 7–20 days after transplantation (Tables II and III). In nine patients blood flow was measured in the renal artery just before closure of the operative incision, using an electromagnetic flowmeter (Table II).

Three of the recipients had hypertension before transplantation. In two the BP became normal immediately after transplantation (Table IV). In all recipients bilateral nephrectomy was done at the time of transplantation. In connection with the opening of the vascular anastomoses 200 mg mannitol was infused. Immunosuppression consisted of azathioprine and prednisone. Azathioprine administration began 3 days before transplantation in a dose of 3 mg/kg b.w./day. Prednisone therapy was initiated 2–3 days posttransplant with 300 mg daily. This dose was reduced during the course of 5 weeks to 30–40 mg/day. Azathioprine administration was continued in a dose of 3 mg/kg/day.

In three patients (nos. 81, 86, 88) moderate rejection episodes were seen 4–5 days after transplantation. It was possible to control rejection on intensification of steroid treatment. In two patients (nos. 89 and 93) severe rejection was seen which resulted in destruction of the graft. In the remaining nine patients the postoperative course was without complications.

Table I *Donor kidney function before neph ectomy*
All clearances are corrected to standard body surface (ml/min/1 73 m²)

Donor no.	Age (y)	Sex	C_{In}	C	C_{Cr}	C_{PAH}	C_{OIH}	FF C_{In}/C_{PAH}	C_{In}/C_{OIH}
74	46	♀	85	89	102	457	437	18 6	20 4
76	48	♂	92	94	108	394	411	23 4	22.9
77	49	♂	74	83	107	342	343	1.6	24.2
78	50	♂	119	113	135	518	402	23 0	28 1
79	42	♂	101	82	134	771	689	13 1	11 9
81	44	♂	98	101	88	667	633	14 7	16 0
84	47	♂	105	119	105	36[illegible]	396	29 0	30.1
86	60	♂	115	128	118	572	492	20 1	26 0
88	52	♀	87	87	87	371	352	23.5	24 7
89	53	♀	53	6[illegible]	75		258		24.0
91	19	♀	91	104	102		489		1.3
93	60	♀	100	101	93		384		26.3
95	40	♀	89	102	135		523		19.5
100	25	♀	94	111	97	671	57[illegible]	14 0	19 4
Average			93 1	98.3	106	513	456	20 1	22.5
S D			16.5	17 1	18 7	152	114	5 1	4 8
Coeff of corr			0.84	0.37		0 96			

METHODS

Time of ischemia was determined as the time from disconnection of ERBF in the donor kidney until ERBF had been reestablished in the recipient. Immediately after removal from the donor the kidney was cooled by perfusion with Perfudex and kept at 4°C until the vascular anastomoses were finished and circulation in the kidney was restored

Immediately before closing the operative incision the flow in the renal artery was measured with an electromagnetic flowmeter (Medicon Microflo) Simultaneous inulin [125]iothalamate creatinine and I-hippuran clearance (C_{In}, C_{Ial}, C_{OIH}) studies w re performed 0– hours postoperatively in 14 recipients, and in 12 PAH clearances (C_{PAH}) were studied as well (Tables II and III) The results from the clearance studies in 9 recipients, carried out using a constant infusion technique as earlier described (11), were compared to the results obtained in the electromagnetic flowmete studies in the same 9 recipients (Table II) All patients received parenteral 0 88 % sodium chloride infused at a rate of 100–250 ml/hour Blood sugar was normal and there was no glucosuria. Follow-up studies were made between –6 and 7 20 day posttransplant (Table III) I the first and second study all recipient had ureta catheter while voiding took place at the bed ide in the last sale

When comparing the donors and recipients w chose to relat graft functio to the donors preoperati bilateral function In T ble II ERBF is calculated from the C_P and C . For calculation of p-values Spearman test and W lc xon s rank-sum test were sed

Table II *Recipi nt kidney function after renal transplanta ion*
Clearances are corrected t standard body surface (ml/m n/1 7 m²)

Pat. no	Age (y)	Time of ischemia (min)	EL flow meter (I)	C_{PAH}	C_{OIH}	ERBF C_{PAH} (II)	ERBF C_{OIH} (III)	ERBF (II)/(I)	ERBF (III)/(I)
74	14	41	450	201	222	272	300	0.63	0 70
76	47	45	490	290	299	387	398	0 86	0 88
77	41	53	420	170	10	250	309	0 60	0 74
79	47	50	500	296	326	405	447	0 81	0 89
81	47	49	1 000	322	406	470	597	0.47	0.59
	36	66	1 200	556	628	713	805	0 59	0 67
[illegible]	28	49	625	294	308	387	405	0 62	0 65
89	17	45	500	196	316	239	395	0 49	0.79
100	[illegible]3	57	390	168	198	233	275	0 67	0 68
Average	33	51	608	277	323	372	436	0 63	0 73
S D		7 5	293	1 1	1[illegible]	154	168	0 13	0 10

Table III *Recipient kidney function after transpl ntation*

Clearances are corrected to standard body surface (ml/min/1 73 m²)

Pat. no	Age (y)	Sex	Time of ischemia (min)	Urine production after (min)	Time after RAT	Proteinuria (g/24 h)	C_{in}	C	C_c	C_{PAH}	C_{131I-H}	FF C_{in}/C_{PAH}	FF C_{in}/C_{131I-H}
74	14	♀	41	1	1 h	3 7	27	26	33	201	222	13 5	11 7
					2 d.	0.6	50	48	61	205	776	4 4	17 4
					9 d.	0.0	40	40	76	295	41	13 5	16.6
76	46	♂	45		1 h	2.1	49	45	50	790	299	16.4	15 0
					3 d	1 1	44	42	57	26.	253	16.8	16 6
					9 d	0.5	78	78	95	377	394	.3 8	19.8
77	41	♂	53	2	1 h	7.5	33	33	34	170	10	19 4	16 4
					3 d.	.0	48	55	57	120	177	38 4	31 1
					9 d.	0 5	65	68	87	349	330	18 6	23 4
78	45	♂	67		1 h	8.0	46	40	40	197	.53	.3 4	15 8
					2 d.	1.2	65	48	81	304	344	1 1	19 8
					9 d	1 1	66	68	74	342	306	19 3	22 2
79	47	♂	50		1 h	3 8	39	42	75	796	326	13.	12 9
					6 d.	4.2	57	65	75	446	405	10 6	16.1
					20 d	0 0	66	77	90	429	413	15 4	18 7
81	47	♀	49	3	1 h	9.0	40	34	39	322	406	1 4	8 4
					5 d.	4.0	34	34	56	173	325	19 7	10 5
					20 d	0 0	49	57	61	371	268	15 3	19 4
84	53	♂	63	5	1 h	1.9	45	47	44	209	40	21 5	17
					9 d.	0 0	64	63	77	295	306	1 7	20.5
86	36	♀	66	2	1 h	6 6	80	59	70	556	628	14 4	9 4
					2 d.	.0	55	51	64	474	995	13 0	8 6
					20 d	0 9	68	76	93	598	547	11 8	13 9
88	28	♀	49	1	1 h	6 6	47	42	52	794	308	16.0	13 6
					2 d.	1 7	40	43	48		298		14 4
					10 d.	0 0	46	45	90	204	47	22 6	18 3
89	17	♀	45	3	1 h	5 1	28	28	38	196	316	17 0	8 9
					3 d	1 4	34	32	45	362	328	9 4	9.6
91	22	♂	48	3	1 h	8 6	42	38	41	777	329	15 6	11 5
					2 d	0 9	66	67	76	258	373	25 6	18 0
					9 d	1 0	88	90	93		436		20 6
93	1	♀	57	3	1 h	1.8	3	31	37		.58		12 6
					2 d	0 4	12	11	17		84		1 6
95	14	♂	45	3	1 h	2	50	39	57		312		1 5
					3 d.	0 9	87	95	95		472		20 1
					8 d	1 1	54	57	76	546	390	9 7	14 6
100	23	♂	57	2	1 h	3 4	34	30	34	168	198	20 3	15 1
					d	1 3	90	49	59	288	258	17 8	19 0
					9 d	0 5	65	59	106	362	312	18.0	18 9
Average	32		52.5		1 h	5.0	42 5	37 8	45 7	264	307	17 0	13 0
					–6 d	1 7	50 0	49 2	60.5	284	338	19 7	16 4
					7–20 d	0 8	6. 4	64 4	81 5	371	349	17 4	18 9
S D			8 3		1 h	7	13 3	8 5	13 1	107	108	3 5	8
					2–6 d	1.2	18 8	20 0	19 3	105	136	8 5	5 9
					7–20 d.	1 4	13 5	14 7	15 7	115	88	4 6	8

RESULTS

The 14 donors who made up a normal tudy material had a mean C_{in} of 98 3 ml/min and C_{131I-H} of 546 ml/min with FF of 22.5 (11) (Table I)

Measurement using an electromagnetic flow meter 9 recipients (Table II Fig 1) revealed the mean blood flow to be 608 ml/mm (range 350–1 200) ERBF measured by C_{PAH} nd C_{131I-H} wa 377 ml/min (range 223–713) and 436 ml/min

Table IV *Recipient blood pressure (mmHg) before and after transplantation*

Pat. no	Before transplantation	Days after transplantation 0–1	2–6	7–20	Antihypertensive treatment before RAT
74	110/70	120/80	125/80	130/80	+
76	125/80	130/70	150/70	150/70	−
77	140/110	190/120	140/110	140/100	+
78	135/60	160/80	160/90	150/90	+
79	190/115	170/90	170/110	155/95	−
81	130/80	140/90	150/90	140/95	−
84	60/110	80/90		160/90	+
86	145/90	140/70	135/85	150/90	−
88	140/90	140/90	150/80	160/100	+
89	110/60	95/40	135/75	140/90	−
91	150/90	155/70	190/90	135/80	−
93	160/100	160 100	170/100		−
95	130/80	150/100	130/95	170/90	−
100	130/70	130/80	140/85	1.5/75	−
Average	137/85	141/82	147/89	147/88	
S D	16/18	21/20	14/12	13/10	

(range 275–805) The relationship between flow measured by C_{PAH} and electromagnetic flowmeter was 0 63 and between flow measured by $C_{131\ I-h}$ and electromagnetic flowmeter it was 0 73 There was a significant positive correlation ($p<0\ 001$) between flow measured with the electromagnetic flowmeter and ERBF calculated on the basis of C_{PAH} and $C_{131\ I-h}$

Three clearance studies were carried out in 14 recipients from immediately posttransplant to 20 days after operation (Table III Fig. 2) Ischemia time was relatively constant at 52.5±8.3 Urine production took place immediately after opening of the vascular anastomoses in all cases C_{In} C_{Io} and C_{Cr} 0–? hours after transplantation were found to be less than half of the donors preoperative bilateral values viz. 45 38 and 45% respectively The 2–6 days posttransplant values were 50 48 and 54% of the donors alues In the third study GFR had increased to 63 64 and 71% of donor values At the time of the first two studies there was a good correlation between C_{Io} and C_{Cr} ($p<0\ 001$) In the third study C_i had increased most,

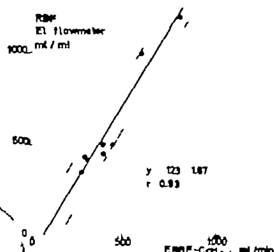


Fig 1 ERBF calculated from [131]I-hippuran learance compared to that measured by electromagnetic flow meter

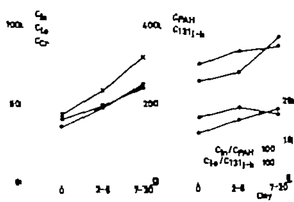


Fig 2 Inul [125]Iothalamat creatinine, PAH and [131]I-hippuran clearance values and filtration fractions i the three tudies

but the correlation to C_{in} was still positive ($0.025>p>0.01$). C_{in} and $C_{131_{I-h}}$ were 54 and 67% respectively of donor clearance in the first study 44 and 67% in the second and 69 and 73% in the third

In the first two studies $C_{131_{I-h}}$ showed higher values than C_{PAH} the $C_{PAH}/C_{131_{I-h}}$ ratio being 0.85 ± 0.09 and 0.82 ± 0.21 In the third investigation the $C_{AH}/C_{131_{I-h}}$ ratio was 1.05 ± 0.14 There was a positive significant correlation between C_{AH} and $C_{131_{I-h}}$ in the three investigations ($0.005>p>0.001$ $0.05>p>0.025$ $0.005>p>0.001$) and a positive non-significant correlation between the donors and the recipients clearance values Proteinuria was 5.0 1.7 and 0.8 g/l in the three studies Serum albumin was constant at values of 3.59 ± 0.49 3.51 ± 0.53 and 3.69 ± 0.39 g/100 ml The FF $C_{in}/C_{131_{I-h}}$ *increased significantly* ($0.025>p>0.01$) based on values obtained in the first and third studies (Fig. 2) In all studies FF $C_{in}/C_{131_{I-h}}$ was significantly lower than the donors FF value ($p<0.001$ in the first study and $0.025>p>0.01$ in the third) In the first two studies the FF C_{in}/C_{PAH} showed higher values than the FF $C_{in}/C_{131_{I-h}}$ contrary to the proportion in the third study

DISCUSSION

Fourteen donors with normal mean values for GFR, ERPF and FF were studied (11) At transplantation a healthy kidney was used as verified by functional studies and renal arteriograms As has been shown *previously the function of the* donors remaining kidney increased compensatorily in all cases (11) In association with transplantation the circulation was interrupted for an average of 5..5 min

The first study was performed immediately after transplantation of the denervated kidney in its new surroundings Measurements of blood flow in the renal artery using an electromagnetic flowmeter revealed a higher flow value than demonstrated by C_{PAH} and $C_{131_{I-h}}$ performed immediately after transplantation. There was a good correlation between the results obtained with the two methods (Fig. 1). These results are not in greement with those obtained in animal studies where almost identical values have been found with the two methods (1). Ischemic damage to tubular cells may explain our observations since extraction of PAH and hippuran has been shown to decrease immediately after transplantation giving extraction ratios of 0.72/0.94 and 0.53/0.70 (6) According to our studies I-hippuran extraction is not less than PAH extraction, since the $C_{131_{I-h}}$ value was higher than that of C_{AH} at the time of the first study Three determinations of ERPF showed very little change in values Values within the lower range of normal were found 7–70 days posttransplant. At the time of the first two studies the ratios $C_{PAH}/C_{131_{I-h}}$ were 0.85 and 0.82 but in the third study a ratio of 1.05 was found We have shown previously that C_{AH} exceeds $C_{131_{I-h}}$ by about 13% in kidney donors (11) and by 0.5% in patients w th renal diseases (12) In four patients with the nephrot c syndrome C_{PAH} was found to be 7% lower than $C_{131_{I-h}}$ These adverse ratios for $C_{AH}/C_{131_{I-h}}$ might be due to a higher degree of protein-binding capacity of $C_{131_{I-h}}$ than of PAH Serum albumin however did not vary in the three studies At the time of the first study severe proteinuria was present, whereas proteinuria at the time of the two following studies was slight. The urinary content of [131]I-hippuran was independent of whether the protein present was eliminated by sedimentation or centrifugation. Thus we have no explanation for these observations of the adverse $C_{PAH}/C_{131_{I-h}}$ ratios but similar results ha e earlier been published by Kou tz et al when investigating renal transplants in the early postoperative course (6)

A reduced GFR revealed at determination immediately after transplantation, resulted in *significant reduction of FF During the course of* the first 70 days the FF increased in patients not evidencing graft rejection This change in FF in association w th transplantation was independent of the recipients BP before transplantation (Table IV) In 13 patients normal BP was measured during the entire period of study Previously a significant correlation between serum albumin concentration and FF has been demonstrated in patients with renal diseases (1) A relationship between serum albumin concentrations and values for FF cannot be demonstrated the present study since serum album n concentrations were constant. In two patients (nos 89 and 93) whose renal grafts were destroyed because of severe acute rejection during the first 10 posttransplant days much lower value for FF was found at the time of the second study This change FF has

been well demonstrated in association with a well defined ischemic period and rejection This was also manifest in the second phase of the renogram which is an expression of the balance between rate of uptake and elimination of ^{131}I-hippuran in the kidney the rate of uptake in these situations compensating for the rate of elimination (2 3 9) We have no explanation of this but intrarenal oedema affecting the glomerulus and causing a reduction in the tubular flow could be the cause (9)

Comparison between donor and recipient values for GFR showed that clearance values in the recipients reached 67% of the donors otal preoperative value within 20 days after transplantation. The recipients clearance increased steadily between the study periods ERPF was found to correspond to 70% of donor values during the whole study period There was a positive non-significant correlation between donor and recipient clearance values

Study of a transplanted kidney from a closely related donor where ischemia time was short and well defined revealed characteristic changes in GFR and ERPF The ERPF reached a value corresponding to 70% of the donors bilateral preoperative ERPF during the course of a few hours whereas the GFR reached 70% of the donors GFR within 20 days posttransplant and thus the FF approached the normal range during this period In spite of the relatively high values for GFR and ERPF ERBF averaged only 73% of blood flow measured in the renal artery

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RENAL ARTERIOVENOUS FISTULA—A CAUSE OF HEART FAILURE

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Abstract Tw cases of renal arteriovenous fistula ar reported in which heart failure was major symptom One patient had auricular fibrillation and suspected mitral alv insufficiency She had observed thrill over her left flank and large renal arteriovenous fistula, probably congenital was disclosed Blood flow through the fistula was measured by dye-dilution tech ique and est mated to be ..8 l/min Cardiac output preoperatively was 6 8 l/min, after nephrectomy 4 3 l/min. Heart siz diminished from 690 to 450 ml/m² BSA The ther patient had a large renal carcinoma and considerable arteriovenous shunting in the tumou Her heart size d minished from 770 to 540 ml/m² BSA after nephrectomy and her clinical condition improved About tw months after operation she died of acut pulmonary oedema.

Renal arteriovenous fistulae previously thought to be very uncommon have been reported more frequently in recent years (5 7) They may cause hypertension due to ipsilateral renal ischaemia cardiomegaly and other sign of heart failur and macroscopic or microscopic haematuria w th or without flank pain

W recently observed two cases of renal arteriovenous fistula in which cardiac failure was major symptom Special haemodynamic tudies were performed in one of these patients

CASE REPORTS

Case 1

The patient was 54-y ar-old woman who had observed irregular heart rhythm for more than f ve years Increased weariness after exertion for about one year and some los of weight. She had physical signs of mitral incompetence and underwent haemodynamic investigation and left ventricular angiography Regarded as nf t for operation she was readmitted to her local hospital and given disablement pension At follow up she told that she had felt thrill over her left flank, and trong bru t could now be heard over her upper abdomen and left flank Abdominal aortography disclosed large central arteriovenous fistula in her left kidney

She was admitted to the Department of Urology University Hospital Lund for operation. Left nephrectomy was performed At the operation th left renal artery was found to be about 15 mm wide and tortuou and the left renal ein was over 35 mm in diamete

The postoperative course was uneventful Her general condition improved and signs of heart failure disappeared even if trial fibrillation persisted.

Case

The patient w a 77-year-old woman complaining of fatigue intermittently for tw months and some eight los She had als observed macroscopic haematuria She was examined at her local hospital and was found to hav pretibial pitting oedema and cardiac enlargement Abdominal aortography disclosed Large right renal tumou with considerable arteriovenous shunting

The patient general condition was poor and she received the suggestion of an operation ery reluctantly A the massive arteriovenous shunting in the tumour considered an important cause of her poor condition radical operation was however strongly advised At the oper ation the right renal artery was found to be of about 15 mm xternal diameter and the renal ein simila in size to the ena cav A accidental lesion to the renal ein ga bleeding of about 7000 ml Right nephrectomy as performed

The general condition improved considerably after oper tion, but she died about tw months later of acute pulmonary oedema

METHODS

In case 1 determination of cardiac output perf rmed by dye-dilution technique 6 months preoperatively and 10 day postoperatively using bromsulphalei as indicator (..3) Selective renal angiography was performed u percutaneous femoral artery catheterization (18) At the time of renal angiography catheter was introduced also into the left renal ein, u the femoral ein. Bl d flow through the renal artery w determined with Card green (14) Calculation of blood flow through the fistula as done according to Ekelund et al (6). At the same t cardiac



Fig 1 Selective angiography of the left kidney in case 1 Huge arteriovenous fistula in middle part of the kidney The main renal artery has a diameter of 15 mm and the renal vein (→) a diameter of 25 mm. Contrast filling of the ovarian vein (⟶).

output was determined using the same catheters and the same indicator

^{131}I-RIHSA (3 μCi) was used for measurement of plasma volume 4 days before and 10 days after the operation Total glomerular filtration rate was estimated by using ^{51}Cr-EDTA with single injection technique (2) 11 days before and 3 weeks after nephrectomy Isotope renography was performed using standard techniques, as well as i. v. urography

In case 2 abdominal aortography was performed via percutaneous femoral artery catheterization. Heart size was calculated from standard chest films in antero – posterior and lateral projections.

RESULTS (Table I)

Case 1

I v urography demonstrated equally sized kidneys with normal excretion of contrast medium The kidney pelvis on the left side was deformed

Selective angiography of the kidneys revealed a huge arteriovenous fistula in the left kidney (Fig 1) The renal artery had a diameter of 15 mm Rapid passage occurred to the renal vein which was much widened to the ovarian vein and to the inferior vena cava.

Mean blood flow through the left kidney was 3.2 l/min (normal mean value for healthy females 434 ml/min) (8) The mean flow through the fistula was calculated to be 2.8 l/min.

Isotope renography showed normal curves on both sides At occlusion of the left renal artery during operation BP increased from 160/69 to 185/98 mmHg but returned to the original levels within a few minutes Pulse rate did not change

Case 2

Renal angiography showed a large malignant tumour Contrast medium from the 12 mm wide renal artery rapidly filled the 30 mm wide renal vein

DISCUSSION

Heart failure is one of the most important complications of arteriovenous fistulae The degree of influence upon the heart is correlated to the size of the fistula opening (9) and inversely correlated to the distance between the heart and the fistula (10) Fistulae localized in the lower half of the body are *said to be more deleterious to the heart than those*

Table I *Laboratory findings*

	Pre operatively		Post operatively
Case 1			
Cardiac output (l/min)	7 4	6.8	4.3
Fistula flow (l/min)	..8		0
Total blood volume (l)	4.6		3.9
Total glomerular filtration rate (ml/min)	74		60
Heart volume (ml/m²)	690		450
Case 2			
Heart volume (ml/m²)	720		540

Six months preoperatively

in the superior vena cava region (17) Renal arteriovenous fistulae are often rather large and this *together with their location in the lower half of the* body and rather close to the heart, would cause a relatively high tendency to circulatory effects. This is in accordance with clinical experience.

Varela (21) gave the first report on an arteriovenous fistula in the kidney The increase in number of renal arteriovenous fistulae reported in recent years is mainly due to the increased use of percutaneous renal biopsy but also to the increased number of renal angiographies performed (7) Among common causes of acquired f stulae are other types of trauma either blunt or sharp renal carcinoma inflammation, and atherosclerosis (15) Congenital arteriovenous fistulae in this region are uncommon they may be solitary or in any type of angiomatous malformation. Some fistulae are classified as "idiopathic" The fistula in case 1 had no certain origin and was probably congenital Arteriovenous fistulae have a tendency to increase in size w th time (13). They may remain silent for many years until they start to grow rapidly and give clinical symptoms (22).

Diagnosis of a large arteriovenous fistula is often made from the present thrill and the bruit. In other cases renal arteriovenous fistulae may be discovered incidentally in the investigation of patients w th hypertension or haematuria. Urography may give a suspicion of renal arteriovenous fistula by showing filling defects calcifications or possibly delayed excretion of contrast medium Renal angiography is the most important method for confirmation of the diagnosis and for demonstration of the anatomical relations *During angiography catheterization also* of the renal vein permits performance of dye dilution study using Cardiogreen® (8) The curve obtained may be typical of an arteriovenous fistula (short appearance time and mean transit time) (6) and thus aids in confirming the diagnosis It may also give an estimation of fistula flow Cardiac output measurement can easily be made at the same investigation It is interesting to note that in case 1 the decrease in cardiac output from the two preoperative to the postoperative values is almost exactly the same as the fistula flow measured at the time of angiography This is in accordance with the view presented by Sumner (19) that the cardiac output is equal to the sum of the normal cardiac output and the fistula flow Compensation for the fistula flow is said to be complete for flows up to 40% of the cardiac output (12). Direct measurements of the fistula flow and of pre and postoperative cardiac output have *to our knowledge not previously* been performed in patients

Arteriovenous fistulae secondary to renal biopsy have a strong tendency to spontaneous healing within the first few months after induction (4 7). In other cases surgical treatment should be resorted to in most cases nephrectomy is performed. In recent years several cases have been reported in which the fistula could be repaired with salvage of the kidney (5 16 20)

In the present cases nephrectomy had to be performed In case 1 the central location of the fistula made conservati e surgery impracticable Ligature of the feeding artery proximal to the fistula, Hunterian ligation, might have been possible. In other parts of the body Hunterian ligation is a poor treatment for arteriovenous fistula (13) as collaterals keep the fistula going. Collaterals may develop even in the kidney (1), which would make simple central arterial ligation an unsatisfactory technique also in the kidney

Of common complications to arteriovenous fistulae heart failure was the only apparent symptom in the present cases The suspected mitral valve insufficiency in case 1 was probably the effect of cardiac dilatation. Cardiac changes as well as ascular morphological changes are not always reversible after repair of fistula. This is illustrated by the outcome in case Even in case 1 although clinical symptoms of cardiac fail re have disappeared and there is a considerable impro ement of the subjective state atrial fibrillation remains.

In the treatment of large arteriovenous fistulae it hould be observed that blood olume is often larger

than normal When the fistula is closed peripheral resistance increases abruptly and the enlarged blood volume may lead to acute pulmonary oedema (3 11)

Continuous monitoring of central venous pressure and of arterial pressure during operation is recom mended and administration of fluids should be done with care The patient should be observed for sev eral days postoperatively and venesection of 500–1 000 ml blood may be necessary In patient I pulse rate did not decrease when the fistula was occluded but there was a temporary rise in arterial pressure.

The glomerular filtration rate after operation was in case 1 reduced relatively little compared to the operative value This could indicate that the nction of the left kidney was reduced On the other and the renal size was equal on both sides and the contrast excretion at urography and the curves registered at isotope renography were essentially normal These findings indicate that renal ischaemia was not present which accords with the absence of arterial hypertension The separate function of each kidney in patient 1 calculated from renography curves and from examination with a scintillation camera (Larsson et al unpublished data) showed the function of the left kidney to be only slightly to moderately reduced as compared to the right kidney The satisfactory preservation of glomerular filtra tion rate postoperatively accordingly most probably indicates good compensatory hypertrophy of the remaining kidney

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RAPID EVALUATION OF CREATININE CLEARANCE

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Abstract Endogenous creatinine clearance, serum creatinine and urinary creatinine excretion have been determined in 368 patients hospitalized with various medical diseases and without known renal disease. The patients were divided into 10-year age groups and the results were analysed for age and sex differences. The average creatinine clearance in males showed a steady decline from 110 to 34 ml/min/1 73 m² from the age group 20–29 to the age group 90–99 years. The mean serum creatinine values did not show any significant variations with age, but urinary creatinine (mg/kg b.wt./24 h) decreased from 23.8 in the age group 20–29 to 9 4 in the age group 90–99 years. In females creatinine clearance declined from 95 to 39 ml/min/1 73 m² serum creatinine was without significant change and urinary creatinine (mg/kg b wt./24 h) decreased from 19 7 in the age group 20–29 to 8 4 in the age group 90–99 years. The finding of considerably decreased creatinine clearance values without elevation of serum creatinine values in the elderly is probably explained by a great reduction with age in lean body mass and thereby in creatinine production. In a group of 106 patients with serum creatinine values between 1 4 and 5 0 mg/100 ml the urinary creatinine (mg/kg b wt./24 h) was not significantly different from that of patients without renal disease A risk of overestimation of glomerular filtration rate seems to exist if serum creatinine is used as the only parameter of renal function in these patients and might lead to intoxication with drugs which are mainly excreted by the kidney The authors suggest a nomogram for rapid evaluation of creatinine clearance in ml/min.

Estimation of serum creatinine has been widely adopted as a screening method for renal function in clinical work (6 7 8 19) A considerable decrease in glomerular filtration rate (GFR) in the elderly was demonstrated by Lewis and Alving (15) and later confirmed by others (4 17 21). However serum creatinine determinations in larger groups of healthy persons have shown no significant change with age (5 13 14) The findings of decreased GFR and unaltered serum creatinine values in the elderly probably indicates a decrease in endogenous creatinine production with age and a fall in urinary creatinine per kg b wt. in the elderly has also been demonstrated by Ahlert et al (1) and by Buhrsu et al (3).

We have studied urinary creatinine serum creatinine and endogenous creatinine clearance in different age groups From these values we suggest a rapid nomographic evaluation of the GFR taking the influence of sex age body weight and serum creatinine into consideration.

MATERIAL AND METHODS

The material includes patients who had been admitted to three medical departments in three hospitals in Copenhagen. The composition of the material is seen from Table I The main diagnosis was essential hypertension in 114 patients and chronic nephropathy in 106 Most of the elderly suffered from arteriosclerotic and cerebral disorders.

In each sex group all the patients were divided into 10-year age groups. Body weight (kg) height (cm), serum creatinine according to the method of Folin. (mg/100 ml) and the 4-hour endogenous creatinine clearance (ml/min) were determined in all patients Many of the elderly had with different indications a catheter à démeure which ensured that the urine was collected quantitatively Urinary creatinine was determined in 3 consecutive 4-hour samples and the mean value was used Only variations in 4-hour creatinine excretion less than 25% were accepted Body surface according to the method of du Bois and urinary creatinine (mg/kg/24 h) were calculated.

RESULTS

Table I shows the results from 149 men and 219 women with serum creatinine values ≤1 4 mg/100 ml and from 51 men and 55 women with serum creatinine values between 1.5 and 5 0 mg/100 ml.

Table I *Results from 149 males and 219 females with serum creatinine ≤1 4 mg/100 ml and from 51 males and 55 females with elevated serum creatinine values between 1.5 and 5.0 mg/100 ml*

Age (y)		B wt. (kg)	Clearance ml/min	Clearance per 1 73 m²	Serum creat. (mg/ 100 ml)	Urinary creat. mg/ 24 h	Urinary creat. mg/kg/ 24 h	Urinary creat. mg/kg/ min×100	n*	Urinary creat. (mg/kg/ 24 h)
Males										
20–29	12	68.4	117±23	110	0.99±0 16	1 625±137	23.8±2.3	1 65	0	
30–39	10	70.9	98±39	97	1 14±0.22	1 520±130	21 9±1.5	1.52	4	21 7±3 7
40–49	32	77.5	98±22	88	1 10±0.20	1 544±421	19 7±3.2	1.37	7	21 9±.4
50–59	37	75 7	88±21	81	1 16±0 17	1 445±252	19.3±2.9	1.34	14	19.8±2.3
60–69	23	73 4	76±22	72	1 15±0 14	1 252±364	16 9±2.9	1 17	13	19 1±3.8
70–79	18	69.5	64±15	64	1 03±0.22	919±13	14.2±3.0	0 99	10	13.2±.7
80–89	12	56.3	45±15	47	1.06±0.25	651±238	11 7±4 0	0.81	1	9.0
90–99	5	67 6	33± 9	34	1.20±0 16	612±188	9 4±3.2	0.65	2	10.7±2.1
Total	149								51	
Females										
20–29	37	58.1	91±19	95	0.89±0 17	1 135±224	19 7±3 9	1.37	0	
30–39	14	60.9	96±25	103	0.91±0 17	1 218±191	20.4±3.9	1 42	4	19 4±4.7
40–49	48	60 1	76±26	81	1 00±0.24	1 056±256	17 6±3 9	1.22	10	16.5±3.6
50–59	34	67.8	74±24	74	0.99±0.26	989±246	14 9±3.6	1.04	11	16.7±.8
60–69	23	65 4	60±15	63	0.97±0.17	871±283	12.9±2.6	0.90	13	15 4±3 4
70–79	27	58.3	49±12	54	1 02±0.23	685±184	11.8±2.2	0.82	1.	10 1±3.9
80–89	32	55.3	41±14	46	1 05±0.22	578±154	10 7±2.5	0 74	4	11.3±1 0
90–99	9	52.4	34± 8	39	0 91±0.12	433±113	8.4±1 4	0.58	1	12.9
Total	219								55	

Patients with elevated serum creatinine.

Evaluation of endogenous creatinine clearance (ml/min) – $\frac{\text{rinary creatinine (mg/kg/min)} \times \text{weight (kg)} \times 100}{\text{serum creatinine (mg/100 ml)}}$

In the males the mean b wt. increased from the age of 20 to the age of 50 and then a gradual decrease was found The mean height in the age group 20–29 years was 178 7 cm and decreased gradually to 168 4 in the age group 90–99 years Changes in the calculated body surface showed ariations from 1 84 m² in the age group 20–29 rs to 1 91 m² in the age group 40–49 years mean body surface then decreased to 1 63 m in the patients aged 80–89 years

Endogenous creatinine clearance in ml/min and corrected to a body surface of 1 73 m² showed constantly decreasing mean values with a gradual fall of about 10 ml/min/10 years The mean serum creatinine values showed only small variations with age and after the age of 30 years no significant differences were found Total urinary creatinine values showed a moderate decrease from the age group 20–29 to the age group 50–59 and thereafter a more pronounced fall was observed. The mean urinary creatinine value in mg/kg b wt./ 4 h showed a constant decrease from the youngest to the oldest age groups and the mean value decreased from 23.8 to 9 4 mg/kg/24 h No significant difference in the mean values of urinary creatinine/kg b wt /24 h was found between patients with serum creatinine values of 1.5–5.0 mg/100 ml and those in corresponding age groups with serum creatinine values below 1.5 mg/100 ml

In general all parameters for the females show values lower than the corresponding values for males The decrease with age in clearance in total urinary creatinine and in urinary creatinine/kg b wt /24 h was of the same degree as in the groups of males Also in the females with serum creatinine between 1.5 and 5 0 mg/ 100 ml the mean urinary creatinine values did not differ significantly from those for the patients with serum creatinine ≤1 4 mg/100 ml

DISCUSSION

Variations in inulin clearance with age have been recognized for many years (4 15 17 1) In pre

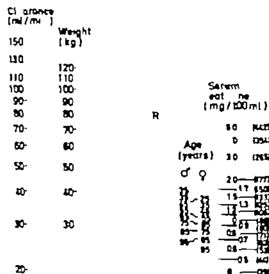


Fig. 1 Nomogram for evaluation of the endogenous creatinine clearance.

Use of the nomogram Connect with a ruler the patient's weight on the second line from the left with the patient's age on the fourth line Note the point of intersection on *R* and keep the ruler there. Turn the right part of the ruler to the appropriate serum creatinine value and the left side will indicate the clearance in ml/min. Serum creatinine values in μmol/l are given within parentheses.

vious reports a reduction in the age group 80–89 years to about 50% of the value in the age group 20–29 years has been found It has also been found that the endogenous creatinine clearance decreases with age (18). The absolute values in our study are somewhat lower but the reduction with age is of the same order Studies of serum and urinary creatinine values in the elderly are few (1 12) and to the best of our knowledge in no previous study have both urinary creatinine/kg b wt. and serum creatinine and endogenous clearance been analysed with special reference to age-dependent changes Studies of very large groups of healthy persons in age groups 20–90 years have shown no significant alterations in serum creatinine values (5 13 14). Our results in hospitalized patients are in agreement with these studies Most textbooks (2 20 22) give values of urinary creatinine/kg b wt./24 h of about 23 mg in males and about 18 mg in females though variations with age are not mentioned

The decrease in urinary excretion of creatinine/kg b wt. in relation to age is probably due to a greater reduction in lean body mass than in total body weight (9 16)

Several investigators have reported decreased urinary creatinine excretion in patients with renal failure especially apparent when serum creatinine exceeds 6 0 mg/100 ml (8 10) However none of these authors mention the age of the patients examined We have been unable to demonstrate a significant decrease in urinary creatinine/kg b wt in patients with serum creatinine values between 1.5 and 5 0 mg/100 ml compared to patients with serum creatinine below 1.5 mg/100 ml and with the same age distribution

Serum creatinine determinations are generally accepted as a screening method for renal function and are often the only parameter used as measurement of GFR in clinical work. Our finding of mean endogenous creatinine clearance values below 50 ml/min in groups of patients older than 70 years but with normal serum creatinine values implies a risk of overestimation of the GFR in these patients. A more accurate knowledge of the GFR has been strengthened through the increasing use of drugs mainly excreted by the kidney This refers primarily to drugs such as digoxin streptomycin kanamycin and gentamicin but might also apply to many other medicaments (11)

On the basis of our mean values of urinary creatinine/kg b wt./24 h we suggest a rapid evaluation of the GFR expressed as endogenous creatinine clearance in ml/min, using the data given in Table I The value for the age group to which the patient belongs multiplied by the body weight in kg and divided by the serum creatinine value in mg/100 ml (parameters which are usually readily accessible in clinical practice) offers an estimation of the patient's creatinine clearance in ml/min with the limitation in correctness shown by the standard deviations of urinary creatinine/kg b wt (Table I). Less precise but quicker is the use of a nomogram shown in Fig. 1

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DOSE REGIMEN OF KANAMYCIN AND GENTAMICIN

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Abstract The half-live of kanamycin and gentamicin have been determined 74 and 49 patients, respectively In patients with normal serum creatinine values the kanamycin $T_{\frac{1}{2}}$ varied from 90 to 538 min and the gentamicin $T_{\frac{1}{2}}$ from 40 to 370 min and in both cases an inversely proportional relationship with creatinine clearance was found. The half-lives of both drugs increased with advancing age reflecting the age-dependent decrease in endogenous creatinine clearance which takes place with unaltered serum creatinine values. This finding invalidates many calculations of drug half-lives based solely upon serum creatinine values Using previously described nomogram it is possible to evaluate a patient s creatinine clearance if the age sex, weight and serum creatinine are known. Calculations of kanamycin and gentamicin half-lives based upon evaluation of creatinine clearance from the nomogram are compared with the measured half-lives. Dose regimens for kanamycin and gentamicin are suggested

Kanamycin and gentamicin belong to the aminoglycoside group of antibiotics which is characterized by a narrow range between therapeutic effectiveness and pharmacological toxicity Neither of these drugs are metabolized the body but eliminated unchanged by the kidneys mainly by glomerular filtration An evaluation of the renal function must therefore be the basis of dose regimens for these drugs Some of the dose schedules given in the literature relate the excretion of kanamycin and gentamicin to endogenous creatinine clearance but this is a rather difficult parameter to obtain quickly in clinical practice (6 9 11).

In some recent papers concerning schedules of treatment with kanamycin and gentamicin serum creatinine has been used as the only parameter of renal function and it has been suggested that the half-lives in hours of these drugs could be estimated by multiplying the serum creatinine concentration in mg/100 ml by three for kanamycin and by four for gentamicin (3) However serum creatinine is of little value in predicting creatinine clearance unless it is taken into consideration that urinary creatinine excretion varies considerably with the sex age and weight of the patient (4 7)

We have previously described rapid bedside method of making an approximate evaluation of the creatinine clearance based upon the weight and the serum creatinine of the patient and upon mean values of urinary creatinine excretion in different age and sex groups (7 11) In the present study this method has been used to calculate the half lives of kanamycin and gentamicin and the calculated half-lives have been compared to measured half-lives Practical dose regimens for the two drugs are suggested

MATERIAL AND METHODS

Kanamycin

The material comprised 74 patients, 35 men (20-9? years) and 39 women (20–90 years) Sixty seven of the patient suffered from functional and arteriosclerotic disorders without known renal diseases and all had serum creatinine values below 1 5 mg/100 ml Seven of the patients had nephropathy of various origin with slightly elevated creatinine values ranging from 1 5 to 3 mg/100 ml

Serum and urinary creatinine and 72 hours endogenous creatinine clearance were estimated by standard procedures (Technicon AutoAnalyzer). Kanamycin half life was determined by measuring the serum kanamycin concentration four times at varying intervals beginning 2 hours after an i.m. injection of 500 mg kanamycin sulphate The kanamycin concentrations were determined by the agar cup method using strain of Bacillus subtilis. In 22 patients the kanamycin determinations were carried out twice on different days on the same serum samples. The standard deviation of the differences between double determinations of half-lives as 16.7% of the mean half-life To evaluate the intraindividual variations in kanamycin half-life repeated de

Table I *Measured kanamycin $T\frac{1}{2}$ compared to $T\frac{1}{2}$ calculated from serum creatinine and t $T\frac{1}{2}$ calculated from estimated creatinine clearance values and distribution volume in three age groups*

Age (y)	20–50	51 70	>70
No. of pats.	13	1	33
Serum creatinine (mg/100 ml)	1 0±0 1	1 1±0._	1.0±0._
Calculated $T\frac{1}{2}$ from serum creatinine (min)	176±21	171±41	200±67
Measured $T\frac{1}{2}$ (min)	107±27	149±49	330±154
Distribution volume (% of b wt.)	16.7±3.0	16.0±6 9	.3 1±6.1
Calculated $T\frac{1}{2}$ from nomogram and formula (min)	117±20	164±35	396±140

$T\frac{1}{2}$ (min) = serum creatinine (mg/100 ml) × 180

$$T\tfrac{1}{2}\,(min) = \frac{V_d \times weight\ (g) \times \log_e}{0.60 \times clearance}$$

terminations were carried out in 10 patients at interval of seven days. The values of the tw half-lif deter minations did not differ significantly (mean values 300± 122 and 282±107 min $p>0.5$) Kanamycin half-life was also determined following i. injection of 900 mg kanamycin sulphate in 19 of the patients. These half-lif determinations did not differ significantly (mean value 268±52 min) from the half-lives determined after i.m. injection (mean value 296±71 min $p>0.1$) The apparent distribution olume of kanamycin was calculated from the extrapolated kanamycin concentration at the time of injection and the total amount of the drug administered An approximate estimate of the creatinine clearance was carried out in all the patients, sing a previously constructed nomogram (7) Using these values of creatinine clearance kanamycin half-life was calculated from the formula

$$T\tfrac{1}{2} = \frac{V_d \times \log_e}{clearance} = \frac{V \quad 0.693}{clearance}$$

V is the apparent olume of distribution nd is the total plasma clearance of kanamycin rding to Orme and Cutler (9) the total plasma clear of kanamycin is about 60% of endogenous creatinine clearance for the whole range of renal function

Gentamicin

The material comprised 49 patient 14 men (70–84 years) and 35 women (18–94 years) All the patients suffered from functional and arteriosclerotic disorders without known renal diseases and ll had serum creatinine values below 1.5 mg/100 ml

Gentamicin half-life was determined like kanamycin half-life but after an i.m. injection of 80 mg gentamicin sulphate The gentamicin concentrations were determined using strain of Staphylococcus aureus The variation of the gentamicin determination and the intraindividual differences of repeated gentamicin half life determinations were of the same order of magnitude as for kanamycin Gentamicin half life was calculated from the above mentioned nomogram and formula using a gentamicin clearance value of 90% of the endogenous creatinine clearance according to the study of Bergan et l (1) ho for all values of creatinine clearance

The data have been subjected to statistical analysis usi g Student s *t*-test applied to a paired comparison

RESULTS

Kanamycin

The measured kanamycin half-lives varied between 75 and 800 min and the endogenous creatinine clear ances between 170 and 13 ml/min An inversely proportional correlation between these two parameters was found In the 67 patients with normal serum creatinine values kanamycin half lives varied between 90 and 538 min and endogenous creatinine clearances between 111 and 27 ml/min

Table I shows the values from the 67 patients with normal serum creatinine values The patients were divided into three age groups The mean values of serum creatinine were practically identical in the three age groups but a considerable decrease in creatinine clearance with age was noted with a mean value of 94±17 ml/min in the youngest age group and values of 75±20 ml/min and 43±1 ml/min in the middle and old age groups respectively

Kanamycin half-lives (min) were calculated by multiplying serum creatinine (mg/100 ml) by 180 as suggested by Cutler and Orme (3) In patients aged 20–50 years the actual measured kanamycin *half-life was shorter than the half life calculated* by this procedure ($p<0.001$) No significant differ ence was found between the measured and the cal culated kanamycin half-lives in the age group 50–70 years ($p>0.05$) but in the older patients the actual measured kanamycin half life was considerably longer than the half-lives calculated from the serum creatinine values ($p<0.001$) The distribution volume (% of b wt) was greater in the elderly ($p<0.001$) compared with the other two age groups Mean values of kanamycin half-lives calculated from the abo e mentioned formula (where V is the mean value of the distribution volume of kanamycin in the three age groups and where creati nine clearance is calculated from the nomogram in every patient) are shown in the last part of the Table It is seen that kanamycin half-life calculated in this way does not differ significantly from the actually measured half-lives ($p>0.05$)

Table II. *Measured gentamicin $T_{\frac{1}{2}}$ compared to $T_{\frac{1}{2}}$ calculated from serum creatinine and to $T_{\frac{1}{2}}$ calculated from estimated c eatinine clearance values and distribution volume in three age groups*

Age (y)	20–50	51 70	>70
No of pats.	12	12	25
Serum creatinine (mg/100 ml)	1.0±0 1	1 1±0.2	1 0±0.2
Calculated $T_{\frac{1}{2}}$ from serum creatinine* (min)	14±22	26_±55	43±44
Measured $T_{\frac{1}{2}}$ (min)	93±76	120±25	216±60
Distribution volume (% of b wt)	15_±7.8	16.2±5.8	4_±5 9
Calculated $T_{\frac{1}{2}}$ from nomogram and formula (min)	9(±13	(44±33	40±57

$T_{\frac{1}{2}}$ (min) = serum creatinine (mg/100 ml) × 40

$$T_{\frac{1}{2}}\text{ (min)} = \frac{V \times \text{weight (g)} \times \log_e 2}{0.90 \quad \text{clearance}}$$

Gentamicin

The measured gentamicin half-l ves varied between 40 and 370 min and creatinine clearance between 1.5 and 29 ml/min. An in ersely proportional relationship was also found between these two parameters.

Table II shows the values from the 49 patients examined. No significant difference was found between serum creatinine values i the three age groups whereas creatinine clearance decreased with age in a similar way as in the kanamyc n material.

Gentamicin half-life was calculated by multiplying serum creat nine (mg/100 ml) by 40 as suggested by McHenry et al (8) and Cutler et al ( ). When these calculated half-life values were correlated to the actually measured half-lives no difference wa found in the elderly patient ($p>0.05$) but in the two younger age groups the calcul ted h lf-lives were considerably longer than the mea ured ($p<0.001$). The distribution olume (% of b wt.) showed higher values i the elderly ($p<0.001$). Gentamicin half-l ves were calculated from the above mentioned formula and nomogram and no significant differences from the actual half l es were found ($p>0.05$).

DISCUSSION

Our findings of inversely proportional relationship between kanamyci and gentamicin half-lives nd endogenous creatinine clearance are n agreement with previous studies (1 5 6). Dose schedules of these drugs must therefore be based upon appropriate estimations of renal function. Creatinine clearance or other methods for the estimation of glomerular filtration rate are usually not available in acute clinical practice. Cutler and Orme (3) therefore suggested that kanamycin half-life (min) could be estimated by multiplying serum creatinine (mg/100 ml) by 180. It has also been suggested that the half-life of gentamicin (min) could be calculated by multiplying serum creatin e (mg/100 ml) by 40 ( ).

In a previous study of 479 patient we ha e demonstrated that urinary creatinine excretion and creatinine clearance decrease with advancing age without any significant changes in serum creatinine (7). This implies that the above mentioned calculations of half-lives suggested by Cutler and Orme with the use of serum creatinine possibly cannot be applied with satisfactory results in all age groups. Our findings of considerable age-dependent variations in measured half-li es of kanamycin nd gentamicin in pite of identical serum creatinine values in the d fferent age groups support this assumption.

Based upon se, we ght and age-dependent aria-tions in urinary creatinine excretion we ha e constructed nomogram from which i s possible to calculate the endogenous creatinine clearance w th a precision of 15% (7 11). Our nomogram has been found alid for all persons with normal serum cre tinine alues and also for patient w th moderately increased serum cre t nine values a the creatinine excretion these patient follow the ame pattern as normal persons. In severe uremia urinary creatinine excretion/kg b wt is decreased causing tendency t o erestimation of the renal function when using the nomogram. The apparent distribution volume for both drugs was found to be increased n the elderly patients in accordance with se ral pre ious tudies (5 6).

If the information from the nomogram and the distribution volume are used the general f rmula relating the half-life of drug t ts clearance the follow ng two equat ons are obtained

$$\text{kanamycin } T_{\frac{1}{2}}\text{ (min)} = \frac{V \times \text{b wt (g)} \quad 0.693}{0.60 \times \text{clearance}}$$

$$\text{gentamicin } T_{\frac{1}{2}}\text{ (min)} = \frac{V \times \text{b wt (g)} \quad 0.693}{0.90 \times \text{clearance}}$$

where 0 60 and 0 90 are the relationships between the clearance of the drug and the endogenous re tinine clearance

I the present study of 1 3 patients we have hown good correlation between the calculated and me sured half-lives of kanamycin and genta-mi in in patients with normal serum creatinine alues The calcul ti ns consider the age-dependent hange in renal function The same accordance was seen in the seven patient with slightly ele vated serum reatinine values

When prescribing each of the two drugs it ha been recommended to give the doses at an interval of three half-li es (10) The usual doses proposed are 7 mg kanamy in and 1–2 mg gentamicin/kg b wt These dose alues and the above outlined interval hould permit adequate antimicrobial ther apy without dangerous accumulation of the drugs

ACKNOWLEDGEMENT

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DRUG INDUCED MALABSORPTION OF VITAMIN B_{12}

VII Mal bs rption of B_{12} during T eatment ith Pot ssi m Citrate

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Abstract T lve of 40 heart patients on medication ith potassium citrate had slightly low results (5–9.5%) in the Sch lling test Administration of calcium at the same time did not prevent this harmful effect. The mechanism of the disturbance i more likely to involve slight acidification of the contents of the ileum than binding of the Ca ion by th citrate The disturbance in the absorption of B during medication with potassium citrate is usually so slight that it can cause deficiency of B only in rare cases. Thi finding is more important as a transient cause of a low Schilling test.

The treatment of heart patients with slow-release tablets of potassium chloride has been shown to cause malabsorption of vitamin B_{12} in 30% of cases (3). The mechanism involved i the acidification of the contents of the intestine Potassium citrate is a weaker acid salt than potassium chloride and hence its effect on the bsorption of vitamin B_{12} should be milder

The present paper reports obser tions on the effect of potassium citrate on the absorption of vitamin B_{12}

MATERIAL AND METHODS

The absorption of vitamin B_{12} was studied in 50 male heart patients in a medical ward Patients th pernicious anaemia, fish tapeworm and other know causes of B malabsorption were e cluded Potassium cit rate Kalmiks solution (Star Ltd Tampere) 30 ml three times daily containing 10 8 g of potassium cit rate (97 mEq K)/100 ml, a administered to 40 patient T study the possibility that the binding of calcium ion by citrate might be the mechanism causing malabsorption of B_{12} g of C (100 mEq) in the form of calcium gluconate (4 tablets Calcium Sandoz forte Sandoz A. G Basel) w given to the remaining 10 patients together with the Kalmik (90 ml daily)

A Schilling test with potassium was performed before treatment and on the fourth day afterwards using 1 µg $^{58}CoB_{12}$ as test dose Normal values in ou laboratory are ≥10% in 4 hours, and pathological al ues <5 whereas values between 5 and 10% may be considered lightly abnormal

Measurements of the pH in the mall ntestine ere obtained from 8 patients during potassium medication using radiotelemetric equipment (Telefunken A G Ulm) (?). The accuracy of the method is 0 5 pH unit.

RESULTS

Schilling tests

The Schilling test results for the group of 40 patients before and during medication with potassium citrate are hown n Fig 1 The alue wa slightly abnormal (actually 5.5–9.5%) in 1 patients (30%) nd normal in 28 (70%) In 7 patients (17%) the Schilling test alue decreased to half of the original value or below the average being 13.3% (without potassium) and 5 1% (with potassium)

Ileal pH

The ileal pH was measured n eight patients The values observed were 6._ in one 6.5 in three 7 0 in one nd 7 5 or abo e n three patients The pH of the Kalemiks® mixture was 5 7

Effect of alcium

I three (30%) of the patients who received calcium gluconate in addition to the potassi m citrat the Schilling test value decreased to half of the original alue or below in one patient to as low as 1%

DISCUSSION

An impaired absorption of vitamin B_{12} was observed in 30% of our patients during medication with a solution of potassium citrate The overall incidence of ll t values was thus

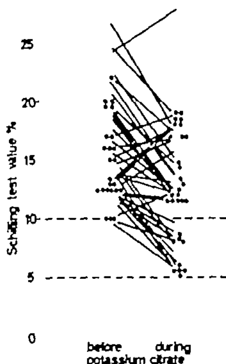


Fig 1 Schilling test values in 40 heart patients before and during medication with potassium citrate

just the same as observed earlier during potassium chloride medication (3) However while the Schilling test value was below 5% in 12% of the patients receiving potassium chloride similar values were observed in only one of 50 patients receiving potassium citrate

In vitro experiments (1) have shown that the intrinsic factor effect on the uptake of B_{12} by ileal homogenate is dependent on the presence of ion and on the pH of the environment. The te ion in the potassium citrate might bind the calcium rendering it insoluble and thus impair the absorption of B_{12} An equivalent dose of calcium to bind the citrate ions did not, however reduce the harmful effect of the potassium citrate Thus it is unlikely that potassium citrate would impair the absorption of B_{12} through the binding of a calcium ion As the pH of the potassium citrate mixture is closer to neutral than that of a 1% KCl solution (5 7 compared with 5 1) no very low pH values for ileal contents were expected The low Schilling test values during KCl medication were connected with pH values below 6 (3) The pH values observed during potassium citrate medication are consistent with the Schilling test values and with the pH of the mixture: both the effect on ileal pH and the absorption of B_{12} are slight.

Impaired absorption of B during treatment with potassium citrate is rather frequent, but it is so mild that it can be expected to cause deficiency of B_{12} only in exceptional cases namely those already on the borderline of B_{12} deficiency for other reasons This finding is more important as a transient cause of low values when interpreting the results of the Schilling test.

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SYSTEMIC LUPUS ERYTHEMATOSUS

Incidence and Manifestations During 14 Years in a Swedish Province

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Abstract Forty-one definite and 25 suspected cases of SLE, 66 cases in all have been investigated at the County Hospital, Halmstad in 1957–71 The occurrence of SLE and related diseases was studied well as the most common clinical manifestations of SLE. The population in the area of uptake was 95 127 in 1957 and 111 707 in 1971 The incidence during 1957–64 was 17.6/year/1 mill inhabitants for definite SLE and 16.2 for suspected SLE. During 1964–71 the incidence of definite SLE was 29 7 and of suspected SLE 12 2/year/1 mill inhabitants. The increasing incidence of definite SLE is probably due to improved laboratory diagnoses, but the possibility that SLE may have become more common cannot be disregarded

SLE is an autoimmune disease which predominantly affects young and middle-aged women and in which a wide variety of organic manifestations occur concomitantly or at different points of time. The course of the disease varies considerably· it is often chronic with numerous re missions and exacerbations, and may vary from at the one extreme spontaneous clinical recovery to at the other fulminant disease with a high mor tality often due to renal failure. The immune re actions of the body are altered considerably There are several points of evidence suggesting that the altered immune reactions are genetically deter mined but in addition to genetic predisposition external trigger factors of various kinds seem to be necessary The possible etiological significance of viral infections in addition to genetic disposition has been much discussed in recent years since virus-like particles have been found in the endothelial cells of the renal vessels the leucocytes and other organs of patients with SLE Animal experiments and investigations of naturally occurring autoimmune SLE-like diseases in for example NZB mice which are also virus carriers support the virus theory The etiology of SLE is however not known and it is probable that several factors are of importance for the oc currence of the manifest disease

Several authors have demonstrated familial aggregation of SLE and similar autoimmune conditions Leonhardt (13) demonstrated an increased occurrence of an increase in polyclonal γ-globulin, high ANF titres and rheumatic factor in lupus patients and their otherwise normal relatives, probably indicating an altered immunological re ponse to endogenic and exogenic stimuli of var ious kinds One finds an increased occurrence of numerous humoral antibodies Complement-bind ing antigen–antibody complexes on the other hand often cause tissue damage Lupus nephritis and vasculitis are often fatal immune complex diseases In addition there is the altered cell-mediated immune reaction which is probably of considerable pathogenic importance in SLE

In addition to the typical clinical symptoms the diagnosis requires various positive laboratory tests such as demonstration of increase in polyclonal γ-globulin of autoantibodies of which the most important are antinuclear factors and of the LE cell phenomenon A further confirmation of the diagnosis can be obtained by recent immunofluorescent techniques and electron microscopic investigations.

At this hospital we have studied SLE during the years 1957–71 with regard to its incidence and various manifestations and to its relation to other collagen and autoimmune d ases

MATERIAL AND METHODS

The population of the area served by the County Hospital wa 95 177 in 1957 and 111 707 in 1971 The study as commenced on 1st July 1957 and ceased on 30th June 1971 From mid 1957 to mid-1964 all patients were diagnosed in the Department of Medicine from mid-1964 to mid 1971 both in the Department of Medicine and the Department of Infectious Diseases. When calculating the incidence the patients resident outside the area of uptak have been omitted.

W ha e mainly applied the diagnostic criteria for SLE presented by Leonhardt (13) and have used Dubol criteria (4) for selection of SLE patients. I addition to the typical clinical signs, numerous positive laboratory find ngs—such as demonstration of increase in γ-globulin autoantibodies, above ll antinuclear factors and of LE cells—were required for diagnosis. Classical patho-anatomical and histological biopsy and autopsy findings supported the diagnosis did also successful treatment with corticosteroids and cytostatics.

Mai ly in accordance with Leonhardt criteria, SLE patients were divided into definite (n=41) and suspected (=25) cases Patients with typical clinical symptoms and typical patho-anatomical findings patients with positive LE cells butterfly xanthema and certai other organic manifestations typical of SLE and patient w th either positive LE cell high ANF titres or butterfly exanthema and typical linical symptoms were allotted t group A definite SLE P tient who did not fulfil all these criteria but had typical findings suggestiv of SLE were allotted t group B suspected SLE

Laboratory d gn sis

γ-globulin in estigations were performed by means of paper lectrophoresis in the Laboratory of Clinical Chemistry at this hospital The upper limit for the normal value was placed at 1 45 g/100 ml 1 recent ears certai immunoelectrophoretic investigations were performed in the Department of Clinical Chemistry Malmo General Hospital and in the Department of Cl nical Chemistry Sahlgren Hospital Gothenburg. ANF titres determined in three laboratories Malmo General ital Sahlgren Hospital Gothenburg, and the Na-Bacteriological Laboratory Stockholm The titre es from different laboratories were not directly comparable

LE cells w re mainly investigated by the Zinkham-Conley method in the Laboratory of Clinical Chemistry at this hospital I addition to the presence of typical LE cells in one and especially several samples, we ha e also onsidered the presence of round haematoxylin bodies of homogeneous type and rosettes since according to the extensiv investigations of Dubois (4) the presence of round haematoxylin bodies and rosettes in several samples is strongly suggestive of SLE

Anemia was defined according t Leonhardt criteria, Hb<11 g/100 ml for men and <10 g/100 ml for women. Leucocyte values below 3 000/mm were considered as suggestiv of leucopenia and fewer than 100 000 platelets/mm of thrombocytopenia, the lower platelet value than in many other materials.

Liver cytology samples were in most cases investigated by Professor N Soderstrom, Department of Medicine Lund. Other biopsy material was mainly investigated in the Institution of Pathological Anatomy Malmö, where also occasional autopsies were performed

Pathological urinary sediments with 5–10 red cells/high-power field in several tests and/or proteinuria in several samples in the absence of concomitant infection were considered suggestive of lupus nephropathy Isolated serum complement investigations were performed in the Department of Clinical Chemistry Malmo General Hospital and the Bacteriological Laboratory Sahlgren Hospital Gothenburg The plasma half-lif (PT½) for INH was determined in the Department of Clinical Chemistry Karol nska Hospital Stockholm

RESULTS

Study of the case report revealed several dis eases which m ght cause diagnostic difficulties with regard to SLE (Fig 1) Rheum toid arthritl was the most common 59 cases in all were found in 1957–71 A combinati n f rheumat id arthritis and LE cells was found in only 3 patients LE cells were not investigated in all cases of rheumatoid arthritl

Cases of scleroderma were distinguished by means of typical skin and muscle biopsy findings and clinical symptoms One patient who had signs of scleroderma on skin and muscle biopsy but subsequently died of generalized lupus disease with typical lupu nephritis was autopsied at Malmo General Hospital Professor J Waldén fr m has provided diagnostic ad ice in several similar interesting cases

Only 5 cases of dermatomyositis were found one with ovarial cancer a the underlying disease

A total of 5 fully developed cases of hydralazine syndrome were found the patients recovering on withdrawal of the drug.

A 60-year-old ma illustrates typical case He had been treated with approximately 300 mg Apresolin daily for 10 years In Feb. 1971 he had developed arthralgia, fever nose-bleed microscopic hematuria, high ESR polyclonal γ-increase high ANF titre positive RA test and pronounced immunoblast nfiltration both n the spleen and liver and muscle biopsy findings compatible with SLE. A few month after withdrawal of Apresolin the patient was clinically symptom-free and there was considerable normalization of both spleen and liver cytology as assessed by Professor N Söderström. Cortisone treatment was not nec

Rheumat arthritis	257
Rheum t rth iti +LE-cell	3
Scle oderm	11
D m tomyositis	8
SLE	86
Hydral l SLE	5
P oc in mid SLE	4
Thrombotic th omb yt pu p	1
I s th mbocyt p p	2
Ho h m l mia	3
Lupoid h patiti	10
Poly t iti nodos	3
Polymyalgia arteritica	48
Goodpa ture's sy d m	2

Fig. 1. Cases of SLE and related diseases hospitalized in the Departments of Medicine and Infectious Diseases, County Hospital, Halmstad in 1957–71

enzyme. Patients who may develop hydralazine lupus syndrome are slow acetylators with low N-acetyl transferase. This patient had long PT½ for INH 5 h and 2 min and is thus a slow acetylator.

Four patients with procainamide-induced lupus syndrome were found. All recovered upon discontinuation of the drug (in 1972–73 a further 4 cases were found).

Only occasional cases of autoimmune hemolytic anemia and idiopathic thrombocytopenic purpura were diagnosed and no case of purpura hyperglobulinemica was seen.

Chronic aggressive ("lupoid") hepatitis was distinguished by means of the clinical picture, laboratory findings and cytology. All these patients except one were women with high ANF titres and positive LE cells. All had antibodies to glomeruli and smooth muscle suggestive of chronic aggressive hepatitis.

Cases of temporal arteritis and polymyalgia arteritica were included in related diseases since these conditions may cause problems of differential diagnosis especially in the early stages of the disease.

The incidence of definite SLE was found to be 17.6/year/1 mill from mid 1957 to mid 1964 and 16.2 from mid 1964 to mid-1971 in the Department of Medicine. The total SLE material selected according to the same principles from the Departments of Medicine and of Infectious Diseases in 1964–71 gave a higher incidence, however 29.7. The incidence of suspected SLE during the first 7 year period was 19/year/1 mill and during the following 7 year period 12.2.

We found approximately the same age and sex distribution as in previous reports with a higher incidence in young and middle-aged women. In our material the mean age at onset of the first clinical symptoms was, however, several years higher than in other reports. The mean age at onset in the Department of Medicine in group A was 46.5 and in group B 46.4 years. In the total material from both departments the mean age at onset in group A was 48 years. Fig. 2 illustrates age and sex distribution at onset of definite SLE.

Delay in diagnosis defined as the interval between the year of onset of symptoms and the year of diagnosis in group A was on an average 3.9, survival after onset 8.6 and survival after diagnosis 4.7 years. Fig. 3 illustrates the pronounced individual variations in the course of the disease.

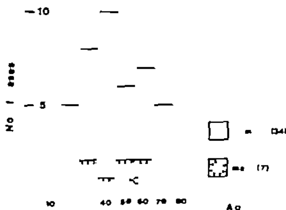


Fig. 2. Age and sex distribution at onset of definite SLE.

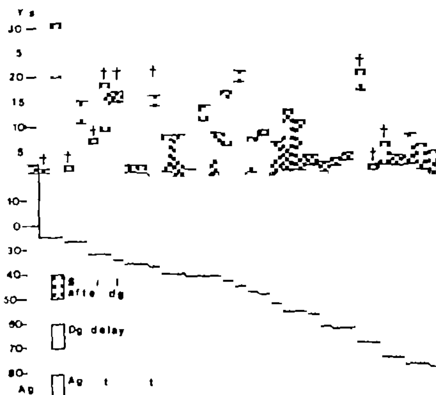


Fig 3 Course of 34 female patients in group A. *Dg*=diagnosis, †=death.

In the women of group A (n=34) The mean age at onset was 48 years the mean delay in diagnosis 4 9 years The mean survival after diagnosis was 4 2 and after onset 9 1 years Nine patients died 5 of them from uremia In group B (n=25) the mean delay in diagnosis was 10 6 years survival after onset 15 9 years and survival after diagnosis 5 3 years Ten patients died, 2 of them from uremia.

Table I shows the percentage occurrence of various clinical manifestations in groups A and B ... figures show many similarities to Leon... .t s larger material from Malmö (13) comprising 57 definite and 52 suspected SLE cases Among the skin symptoms found in the present material was the chronic discoid type of LE exanthema, but acute or subacute skin conditions of morbilliform and scarlatinal type and recurrent urticaria were more common. The typical butterfly exanthema was found in 39% of patients in group A Purpura, which can be caused by thrombocytopenia or vasculitis, was diagnosed in only a few cases as also was loss of hair

Joint symptoms in the form of arthralgia and often myalgia without definite objective findings occurred and acute and subacute polyarthritis with localized swelling of the joints and restriction of movement, involving various small and large joints were also found. Chronic joint changes which could often not be distinguished from rheumatoid arthritis with typical radiographic arthritic and/or typical clinical symptoms, were often found. Lymphadenitis was diagnosed in only 24% of patients in group A

Liver involvement was present in half of the cases Le either pronounced enlargement of the liver and/or pathological liver tests (positive thymol not included) and pathological liver cytology findings Seventeen patients had pathological liver tests usually in the form of raised transaminases raised alkaline phosphatases sometimes raised bilirubin Liver cytological investigations were performed in 11 patients in group A One patient had slight steatosis during concomitant steroid therapy this being a non-specific finding Two patients had very extensive steatosis, one of whom had pronounced enlargement of the spleen with pronounced blast cell infiltrations Four patients had symptoms of active hepatitis with pronounced lymphoblast and immunoblast infiltration.

Table I *Cumulative incidence (%) of commonest clinical manifestations of SLE in the present study and in Leonhardt's material*

	Present study		Leonhardt (13)	
	A (n=41)	B (n=25)	A (n=57)	B (n=57)
Cutaneous LE	78	28	60	35
Butterfly exanthema	39	16	49	15
Purpura	5	0	19	17
Loss of hair	10	4	25	4
Arthropathy	90	80	93	92
Rheumatoid arthritis	29	56	18	35
Lymphadenopathy	24	0	46	25
Splenomegaly	20	12	23	25
Hepatomegaly }	59	20	16	1
Hepatopathy }			7	11
Pulmonary lesions	46	20	60	44
Pleurisy	37	28	70	64
Myocarditis }	32	28	28	15
Pericarditis }			21	19
Raynaud's phenomenon	15	0	25	21
Leg ulcer+phlebitis	17	4	28	10
Sicca syndrome	12	8	11	31
Irido-retinitis	10	4	1	4
CNS lesions	7	8	11	8
Nephropathy	34	28	56	23
Anemia	37	32	63	42
Leucopenia (<3 000/mm³)	22	4	77	56
Thrombocytopenia (<100 000/mm³)	17	4	32	17
Hyper-γ				
(>1.45 g/100 ml)	83	72	90	90
(≥2.0 g/100 ml)	63	24	68	69
LE cells	71	48	75	54
LE cells – RHB rosettes	85	84	–	–
ANF	96	55	–	–
Rheuma serology	44	39	41	66
Positive Coombs test	12	4	18	2
False pos. syphilis serology	5	0	16	15
Fever	78	42	88	92
Drug reactions	34	0	53	31
Remitting course	63	48	91	85

One patient had pronounced bile duct proliferation. (One patient had extensive cirrhosis of the liver at autopsy.)

Chronic pneumonia not responding to antibiotics, pleurisy (often bilateral and recurrent) and various forms of cardiac involvement, above all perimyocarditis, occurred in 46–32% of the patients. In two cases classical endocarditis of Liebmann–Sachs type was found at autopsy. Raynaud symptoms occurred in 15% but no definitely positive cryoglobulin tests were found in this group. Among CNS symptoms found were cranial nerve paralysis, convulsive seizures and mental confusion responding to treatment with cortisone.

Nephropathy was found in only one-third of the patients. In view of the frequent occurrence of concomitant infections and other kidney diseases we included only definite or strongly suspected cases comprising nephropathy with or without nephrosis and fatal glomerulonephritis with uremia. Serum complement investigations were performed in two patients with active glomerulonephritis and uremia and the titres were found to be low. Two patients with lupus nephritis and preuremia or uremia were subsequently admitted to the University Clinics in Lund and Gothenburg respectively.

The occurrence of anemia, leucopenia and thrombocytopenia was low compared to other reports. The criteria for anemia in women was Hb<10 g/100 ml. A large number of women had Hb values between 10 and 11 g/100 ml.

Hypergammaglobulinemia >1.45 g/100 ml occurred in 83 and 72% and in 63 and 4% respectively. γ-globulin was ≥2 g/100 ml. All of these patients had increased polyclonal γ-globulin with the exception of two who had discrete M components of benign type.

One elderly man with familial disposition for joint pains had on admission symptoms suggestive of SLE, bilateral arthropathy, tiredness, loss of weight, moderate enlargement of the liver, anemia, high ESR, typical LE cells in one test, and highly positive ANF titres in three samples. Immunoelectrophoresis showed, however, monoclonal γ-band and IgG L myeloma. Radiographic skeletal changes typical of myeloma developed subsequently. The patient did not fulfil the criteria for suspected SLE and was excluded from the group.

Of the cases in group A 71% and in group B 48% were found to be LE cell positive, most of them investigated according to the Zinkham–Conley method. The figures rose to 85 and 84% when round hematoxylin bodies of homogenous type and rosettes were included. All patients from the Department of Medicine in group A diagnosed after 1964 had positive ANF titres. ANF titres were investigated in 11 of 13 patients from the Department of Infectious Diseases, one of them having a negative titre investigated once and the other ten positive. The incidence of positive ANF titres in the total material from both

partments was 96% In most cases the laboratory concerned considered the titre highly positive and suggestive of SLE Only 60% of the patients in group B had positive ANF titres Most of the patients with negative titres received concomitant corticosteroid therapy The ANF titre was found to fluctuate considerably during the course of the disease One patient with lupus nephritis became ANF-negative during the fulminant phase of the disease during which treatment with azathioprine and cortisone was given Some of the other patients had high titres without signs of active disease.

Different kinds of tests for the presence of rheumatoid factors were used They generally yielded weakly to moderately positive results thus a non-homogenous group showing positive figures in 46 and 39% respectively Only a few cases with positive Coombs test were found in group A and these had definite hemolysis. The figures for other non-specific antibodies and false positive results of serological tests for syphilis performed in only a few cases were low compared to other series Fever and recurrent infections occurred frequently

Many interesting constellations of symptoms were observed Some patients in group B had had symptoms of rheumatoid arthritis for many years and had subsequently been admitted to hospital with refractory pleurisy symptoms of nephritis or hemolytic anemia suggestive of progression to SLE. This group included several other patients with interesting combinations of diseases such as the occurrence of rheumatoid arthritis hyperthyroidism, myasthenia gravis and histamine-refractory achlorhydria as well as LE cells in one and the same patient. Another patient had the e clinical combination and in addition pronunced vasculitis with formation of skin ulcers which healed after treatment with cortisone Group B also included two patients with chronic discoid lupus erythematosus. One of them also had certain manifestations suggestive of SLE, while the other had only this cutaneous manifestation, LE cells and persistent increase in polyclonal γ-globulin. One patient in group A had in addition to rheumatoid symptoms vasculitis butterfly exanthema, leucopenia and LE cells highly suspect chronic lymphatic leukemia. Most of the patients in group B had a more benign and chronic course and the delay in diagnosis was several years longer

Polypharmacy has been widespread. Drug reactions occurred in 39% of the patients in group A. Four had penicillin reactions one anaphylactic shock after injection of penicillin three skin reactions after treatment with sulphonamides one developed a skin reaction after treatment with iodine and one after treatment with acetyl salicylic acid. Another patient developed a skin rash, possibly due to the colouring matter in Deltacortril® tablets and one had suspected eryhromycin erythema. In one case SLE developed shortly after treatment with gold. We assess remission of the disease to have occurred in slightly more than half the cases. These figures are difficult to judge however

As regards therapy salicylates were given to practically all patients Antimalarials were given to several patients with joint symptoms and to patients with chronic discoid lupus erythematosus Cortisone was given to 36 of the 41 patients in group A 18 required continuous treatment, usually with low doses 5–7.5 mg Prednisolon® daily A dramatic improvement was noted in connection with large doses of cortisone in patients with severe cardiopulmonary manifestations CNS syndrome hemolytic attacks and thrombocytopenia. One woman in group B with pleuropneumonia and pericarditis of SLE type with much impaired general condition and high fever recovered spontaneously and was symptom-free for several years except for raised ESR Azathioprine was given together with cortisone to only four patients, three of whom had lupus nephritis One patient improved with reduced proteinuria and normalization of serum complement. In another patient with nephritis and uremia, treatment with azathioprine and cortisone brought only a transient improvement and in a third patient no improvement was observed.

Nine patients in group A died five from uremia, one from leucopenia and fever one from furunculosis in connection with fracture of the femur one from gastrointestinal bleeding and one from myocardial infarction. Ten patients in group B died two from uremia, two from heart failure due to perimyocarditis one from myocardial infarction, one from complete AV block and septic arthritis one from gastrointestinal bleeding one from breast cancer with metastasis and one from generalized fungal infection. One patient died at home possibly from bleeding gastric ulcer

DISCUSSION

The present study shows considerable similarities regarding clinical and laboratory findings and course to previous SLE series, especially the investigations published by Leonhardt (13). In the present study the definite cases of SLE were defined according to principles generally accepted by many authors and confirm the clinical entity of definite SLE. Many borderline cases were found, however, between suspected SLE and related diseases, some of these later showing typical signs of definite SLE. Leonhardt (14) demonstrated the most important manifestations of SLE with regard to incidence in his own and five other series. He found considerable variations in incidence due to differences in the primary patient series, in criteria for selection and in definitions of symptoms such as leucopenia, etc. The frequency of SLE compared to that of related diseases in this study shows many similarities to the investigations of Dubois (4) and Kurland et al. (10).

In the present study three patients were found with rheumatoid arthritis and LE cells. This combination is considered to be a fairly benign condition, very rarely progressing to active lupus disease. Lupus nephritis according to Dubois (4) has however been found in renal biopsies among patients in this group.

Patients with hydralazine syndrome could be distinguished from SLE patients by recovering on withdrawal of the drug; besides, these patients belong to the slow acetylator group.

Four patients had procainamide-induced lupus syndrome, all of whom recovered upon discontinuation of the drug. Henningsen (8) found in a prospective study that more than half of the patients given procainamide developed positive ANF, often with increasing titres. Some of these may progress to lupus syndrome. Henningsen therefore recommends ANF control at intervals of 2–3 months. Serious kidney injuries have not been reported either in procainamide or hydralazine-induced lupus syndrome.

Among the SLE manifestations nephropathy was found in only one-third of the patients. The incidence of lupus nephritis was probably considerably higher, but in only a few cases were biopsies and thorough autopsies performed. The incidence of nephropathy is higher in most other SLE series published. Thus, according to Pollak et al. (19), renal involvement is found at autopsy in at least 75% of patients with SLE. Lymphadenopathy was diagnosed less frequently in this material than in many others, probably due partly to inadequate clinical diagnosis. Hepatopathy was commonly found in this study, occurring in 59% in group A, probably due to cytological and repeated laboratory investigations. Anemia, leucopenia and thrombocytopenia were not so common in this as in Leonhardt's study. Hb concentrations less than 10 g/100 ml in females were considered anemia. Hb values between 10 and 11 g/100 ml were very often found, however. Many patients had leucocyte counts just above 3 000/mm and a platelet count just above 100 000/mm.

LE cell positive cases increased from 71 to 84% in group A and from 48 to 84% in group B when round hematoxylin bodies of homogenous type and rosettes were included. According to Dubois' extensive investigations (4) the presence of round hematoxylin bodies and rosettes in several samples is highly suggestive of SLE. False positive syphilis serology was found only in two cases. Unfortunately this test was performed in only a few cases.

Polypharmacy has been widespread and resulted in many side-effects; one patient developed lupus shortly after treatment with gold; exacerbation of lupus disease after gold therapy has been reported in other series. Seven patients had side effects to antibiotics. Presumably one ought to be more restrictive in treatment of these cases with sulphonamiedes and pecillin and indeed drugs in general.

As regards therapy, salicylates were given to practically all patients, one of them showing skin reaction in connection with acetyl salicylic acid treatment. Antimalarials were given without any side-effects and most of the patients were given cortisone with often dramatic improvement; only four patients received azathioprine.

Approximately the same age and sex distribution was found in this study as in previous reports, with a higher incidence in young and middle aged women. In this study the mean age at onset was, however, several years higher than reported by others. In Leonhardt's material, for example, the mean age at onset in group A was 36.6 years and in group B 39 years as compared to 48 and 46.4 years in the present study. Larsen (11) found that 70.8% of the women first fell ill between 20

nd 50 years of age and the disease was diag nosed between 40 and 60 years in 70.5% of cases

The delay in diagnosis in group A in this study was 4 9 years about .6 years shorter than that reported by Leonhardt. In group B the delay in this study was 10 6 years about 3 1 years longer than in Leonhardt s material

The mean survival time after diagnosis was 4 ? years in group A and 5.3 years in group B How ever the follow-up time is short in the present study in several cases only a few years As ex pected there was a high mortality due to lupus nephritis of 66 SLE patients 17 died six of them from uremia Leonhardt (16) estimated the long-term prognosis for SLE patients and reported a 5-year survival after diagnosis in 70.3% and a 10-year survi al in 51 3%

It is noteworthy that the incidence tends to ncrease when the cases from the Departments of Infectious Diseases and Medicine are added together it reaches 29 7/year/1 mill inhabitants (mean incidence during the years 1964–71) The population increased during the same period from 99 000 to 111 700 Leonhardt found the incidence between 1955 and 1961 to be 10 3 in Malmö with 50 000 inhabitants The incidence from Scania ex cept Malmö was 5 2 Larsen (11) found the incidence in central and western Norway to be similar to that in Leonhardt s study varying between 4 1 in 1961 and 10.3 in 1962 His material was collected during the years 1959–63 The increasing incidence was considered to be mainly related to the introduction of the LE cell test. Siegel et al (22) estimated the approximate incidence in New York City since 1955 to be 10/year/ ill inhabitants In a later investigation Siegel (21) reported higher incidence figures In es from New York City and Jefferson County labama in 1956–65 these authors found a total incidence in New York of 18 5 and in Alabama of 9.9/1 mill A considerably higher incidence was found in coloured women 73 4 in New York and 26.5 in Alabama, probably due to ethnic fac tors Among white women in New York City the incidence was 25 7 and in Alabama 10.6 Siegel considers the increasing incidence of SLE to be due mainly to improved diagnostic resources He also found increasing prevalence figures due to earlier diagnosis and improved therapeutic re sources. Conclusions made from the present study the limitations of which are recognized support these opinions

The increasing incidence of SLE is probably due to improvements in diagnosis but the possibility of SLE becoming more frequent, possibly as a result of various environmental factors bac terial and viral infections together with polypharmacy and other chemical and physical triggering factors cannot be excluded

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Table I *Laboratory data before and during treatment with prednisolone and cyclofenil*

After 1/? months the prednisolone dose was reduced to 5 mg/day and the cyclofenil treatment started. After a further 6 months prednisolone treatment was stopped and the cyclofenil dose doubled

	Before treatment	After 2 1/2 mo prednisolone (?0–30 mg/d)	After 6 mo cyclofenil (300 mg/d) + prednisolone (5 mg/d)	After 4 mo. cyclofenil (600 mg/d.)
Hb (g/100 ml)	13 1	13.2	13 7	14 6
RBC/mm³ 10⁶	4.8	4.3	4 4	5 0
WBC/mm	7 000	6 000	7 400	6 000
Platelet 1 000/mm	247	251	250	327
ESR (mm/h)	57	31	29	20
Serum creatinine (mg/100 ml)	0 9	0.8	0.9	0.9
Serum calcium (mEq/l)	4.9		4.8	4.6
Serum albumin (g/100 ml)	3 9	4.2	3.9	4.2
Serum haptoglobin (mg/100 ml)	40	170	155	L5
Serum bilirubin (mg/100 ml)	0 6	0 4	0 6	0.4
Thymol (U)	1 0	0 9	1.5	1 4
Alkaline phosph (U)	1.0	1.2	1 6	1.6
GOT (U)	20	12	56	35
GPT (U)	24	11	72	46
LDH (U/ml)	283	201	290	281
Rheumatoid factor (latex fixation test)	Pos. 1/640		1/320	
Serum γ-globul	Elevated		Elevated	Normal
ANF	Pos. 1/400	Pos. 1/400	Pos. 1/100	Neg.

thritic changes together with the stiffness of the skin resulted in restrictions of head movements and inability to elevate the arms above the horizontal plane.

In Feb. 1973 the patient was admitted to the Department of Medicine In addition to the physical changes X-ray examination revealed reduced esophageal mobility Laboratory investigations showed high ESR, moderate decrease of serum albumin, increased serum haptoglobin level normal liver and kidney functions There were positive titres of the latex fixation test and of antinuclear factor (ANF) (Table I).

Conventional prednisolone therapy was started with ose of 20–30 mg/day Simultaneously intense physiotherapy by daily exercises and hykko-baths were against the arthritic changes After 2 1/2 months ednisolone therapy no subjective or objective provement could be observed and the clinical condition continued to progress. At this time the prednisolone dose was reduced and Sexovid (cyclofenil) treatment was started with daily dose of 100 mg three times daily

After some weeks the patient noticed considerable improvement in serveral respects The affected skin areas became gradually softer less stiff and more elastic, especially in the face, neck and chest. The joints were swollen and painful and the mobility increased After 6 months treatment the beneficial action of the drug and its lack of side-effects led to an increase of the dose to 200 mg three times a day The patient was observed further on the higher cyclofenil dose over 5 months. From May to Nov 1973 the prednisolone dose was kept at a very low level (5 mg/day) and since Nov the patient has received no prednisolone at all

The results of some laboratory investigations are shown in Table I It can be seen that during the 1 year observation period there were no changes in the standard haematological values ESR and the serum haptoglobin levels show a gradual decrease In contrast, the cyclofenil treatment caused a moderate increase in the serum GOT and GPT levels indicating possible effect of the drug on liver cells However despite further uninterrupted cyclofenil administration the transaminase levels decreased again. Recently the liver cells appeared microscopically normal in a biopsy material and the galactose loading test was found to be normal. The titre of rheumatoid factor decreased and the ANF disappeared, possibly due to the corticosteroid treatment

The improvement in the patient s respiratory function over the last treatment period is shown in Table II. It is rather difficult to record small changes in joint function objectively but an attempt was still made to study mobility in some joints especially involved. Table III demonstrates that a certain increase in the mobility of the shoulders, wrists and the small interphalangeal joints had been achieved. The stiffness of the neck diminished and the gaps between the teeth had increased The drug was apparently well tolerated during the whole observation period and apart from the moderate increase in transaminase levels no side-effects were noticed

Table II. *Effect of treatment with 600 mg cyclofenil daily on the respiratory function measured by dynamic spirometry*

MVV maximum voluntary ventilation, PEF = peak flow FEV = forced expiratory volume

		Before dose increase to 600 mg		After 4 mo on 600 mg	
	Normal value	Patient value	% of normal	Patient value	% of normal
MVV (l/min)	149	90	60	118	79
MVV_{m} (l/min)	122	90	74	109	89
PEF (l/min)	580/425	475		560	
Vital capacity (l)	4.8	3 7	77	3 9	81
FEV (l)	3.5	2.8		2 9	

DISCUSSION

There are several factors which make it difficult to evaluate the possible therapeutic effect of a new drug in systemic scelrosis Firstly the generalized forms of the disease are rather rare Secondly the clinical course and symptomatology as well as the character of the pathological changes differ from case to case Thirdly spontaneous periods of standstill and remissions are not uncommon Moreover specific laboratory parameters that might indicate the progress or regress of the disease are lacking

In the present case the observation time was made rather long in order to exclude the probability of spontaneou remission. An attempt was also made to objectively quantitate respiratory function and joint mobility in the affected joints but it is obvious that the pathological change before treatment must be rather advanced to permit the recording of significant improvements There fore in mild cases and in the early phase a suitable biochemical parameter would be needed for evaluation of small therapeutic effects The present case was advanced before treatment and cyclofenil caused measurable improvement of the kin and joint changes as well as of the lung function. However t is also of interest that the laboratory profile became somewhat altered during the treatment. The decrease in some immunological titres and the fall of the serum haptoglobin level may be due to prednisolone administration in the initial stage but the changes appeared in connection with the cyclofenil treatment and seem to be permanent despite a long prednisolone-free period. During the experimental evaluation of cyclofenil however no anti-inflammatory effect was detected (2)

Nothing is known about the mechanism by which cyclofenil acts upon the sclerodermic tissue. It is unlikely that the pituitary-inhibiting properties would play a role in producing the beneficial effects I PSS It is rather possible that this drug, in analogy with conventional estrogens I some way alters the metabolism of collagen and proteoglycans. A variety of such estrogenic action has previously been demonstrated

Table III. *Function of some joints measured before and after cyclofenil treatment*

Until 8 months 300 mg, after 8 months 600 mg daily

	Before treatment	After 8 mo treatment	After 1 y treatment
Maximal elevation of the arms in the sagittal plane	120°	135°	145°
Fist closure: long-finger distance (cm)			
Right	3.0	1 5	1.0
Left	2.0	1.0	0.5
Dorsal flexion of the wrist joint			
Right	5°	7°	10°
Left	5°	10°	15°
Palmar flexion of the wrist joint			
Right	5°	10°	15°
Left	10°	10°	15°
Maximal anteroflexion of the trunk, distance between fingertips and floor (cm)	30	14	15
Maximal dorsal flexion of the head distance between incisura jugularis and lower tooth row (cm)	17	17.5	?0

in animal experiments by many investigators (4 7 9 10 13) Recent studies indicate that the rate of collagen and proteoglycan synthesis in sclerodermic skin is rather well correlated with the severity of the disease (14) Thus incubation experiments using skin biopsy specimens and radioactive precursors at different intervals during cyclofenil treatment seem to be a possible way to evaluate therapeutic effect Such objectively controlled studies have now been started in our department on a larger group of PSS patients Using doses similar to those in the present case cyclofenil might be somewhat estrogenic and inhibit gonadotrophin secretion (2 11 12) Therefore it seems convenient to test further the effects of the drug in a female material where estrogenic side-effects would be of no significance

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RECURRENT ATTACKS OF HEMOLYTIC ANEMIA THROMBOCYTOPENIA AND UREMIA—HEMOLYTIC UREMIC SYNDROME?

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Abstract. The case history of a 3-year-old boy with 6 relapses of hemolytic anemia, thrombocytopenia and uremia is reported. Between the attacks the glomerular filtration rate was normal and there was no proteinuria. The possible relationship to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura is discussed.

In 1925 Moschcowitz (10) described thrombotic thrombocytopenic purpura (TTP) The disease was characterized by fever purpura, hemolytic anemia, neurological manifestations and sometimes uremia. The typical histological lesion was occlusion of the small arteries in multiple organs caused by disseminated intravascular coagulation. Thirty years later Gasser et al (7) reported the hemolytic uremic syndrome (HUS) as a new symptom complex including hemolytic anemia thrombocytopenia, neurological manifestations and severe uremia. The disease was always lethal and autopsy showed bilateral cortical necrosis in the kidneys and dispersed thrombotic microangiopathy *Gasser et al* emphasized that this new syndrome differed from the TTP mainly in the severe kidney damage

The etiology of these syndromes has been widely discussed but remains unknown. Before treatment with dialysis heparin and corticosteroids the course was nearly always fatal The prognosis at present may have changed and total remission has been reported (9)

As regards the prognosis we therefore find it important to report on a case of a 3-year-old boy with total remission in kidney function after six relapses of hemolytic anemia, thrombocytopenia and uremia.

CASE REPORT

A 3-year-old boy was admitted to the hospital in Nov 1971 (Fig. 1) with 2 days history of fatigue, nausea, vomiting, jaundice of the sclerae dark urine, petechiae and ecchymoses on the legs all antecedent by common cold He had a moderate enlargement of the liver and small ulcerations of the tonsils, which both vanished during the hospitalization

Analyses showed Hb 6 g/100 ml platelet count 5 000/mm³ reticulocytes 5% osmotic resistance normal, haptoglobin slightly decreased leucocytes 1 000 mm³ The peripheral blood smears showed considerable degree of anisocytosis and poikilocytosis with numerous Burr cells. Bone marrow aspiration revealed no signs of leucemia. Clotting time 3 ½ min bleeding time more than 10 min, prothrombin 64% All other coagulation factors normal. Serum creatinine 2.6 mg/100 ml BP 115/65 protein content in urine 5 g/24 h. Urine sediment 40–50 erythrocytes 6–8 leucocytes, urine urobilin positive Total serum bilirubin 3.0 mg/100 ml SGOT and SLDH grossly increased, alkaline phosphatases normal Liver biopsy normal, kidney biopsy unsuccessful. The following tests were all negative: Coombs test, antiglobulin consumption test, antinuclear factor LE cells, WR, Paul-Bunnel toxoplasmosis, herpes virus, leptospirosis, influenza complement, adenovirus, streptococcic MG cytomegalovirus, Weil polio, echo virus in faeces. Urine mercury serum lead, copper zinc and iron all normal.

The patient was treated with two blood transfusions and recovered completely within 7 days Kidney function returned to normal and proteinuria vanished.

The patient remained healthy until 10 months later when he was readmitted to the hospital with the same symptoms of dark urine jaundice, fever petechiae and ecchymoses. Laboratory investigations as above. He was treated with blood transfusions, platelet transfusions and furosemide He recovered slowly to normal kidney function and proteinuria disappeared.

Six months later he was readmitted again with symptoms as earlier On this occasion the kidney function was severely decreased and the patient had to be transferred to the Nephrological Department for peritoneal dialysis. Due to the dialysis short treatment with heparin was interrupted. The patient was given prednisone 40 mg/day After two peritoneal dialyses he recovered slowly and the prednisone therapy was withdrawn.

Kidney biopsy examined by Dr C. Brun, showed glomerulonephritis proliferativa, TTP (Mos-

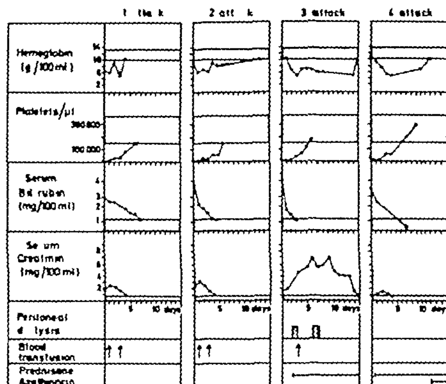


Fig. 1. Laboratory data from the first four hospitalizations.

and is illustrated in Fig. . In addition to the former laboratory investigation on this occasion a consumption coagulopathy was found. Free Hb in serum 161 mg/100 ml in urine 404 mg/100 ml. The antiglobulin consumption test was now positive. Erythrocyte glucose-6-phosphate dehydrogenase activity normal. I urography normal.

Again he remained healthy with normal kidney function and no proteinuria until 9 months later when a new relapse took place with symptoms as earlier. The patient was immediately treated with prednisone 40 mg/day and recovered quickly without need of dialysis. Treatment with azathioprine was started as long-term therapy and simultaneously the prednisone dosage was reduced.

During further follow-up (not indicated in Fig. 1) the patient has had more hemolytic episodes with temporary affection of the kidney function and the azathioprine treatment has now been withdrawn. The kidney function is again normal and no proteinuria remains.

DISCUSSION

The case history of a 3-year-old boy with six relapses of hemolytic anemia, thrombocytopenia and uremia and normal kidney function and no proteinuria between the attacks is presented. Each attack fulfils the typical clinical and pathological features of the hemolytic uremic syndrome but to the authors' best knowledge recurrent relapses after total remission of the HUS have not previously been reported.

The etiology of the HUS is still uncertain. Many authors have discussed the possible relationship to a preceding virus infection. Gianantonio et al. (8) suggest an epidemic appearance and have isolated a conceivable viral agent in 8 patients during the acute stage of HUS. In 36 of 55 patients they found complement fixing antibodies to this virus. Ray et al. (11) demonstrated in 2 patients a possible connection between infection with coxsackie virus B positive serology and the acute stage of HUS. Furthermore in 1970 Ray et al. (17) found serological evidence of infection with enterovirus (coxsackie virus) in 7 of 9 patients with HUS. We have not been able to demonstrate any agent responsible for the outbreak of HUS in our patient but every attack was preceded for a few days by signs of a "common cold".

Other possible etiological mechanisms have been reported, e.g. estrogens (1), drugs (15), endotoxin reactions (Schwartzman's phenomenon) (14), decreased glucose-6-phosphate dehydrogenase activity (favism) (4), sicklemia (3), but the problem is still open for further investigation.

HUS and TTP are commonly considered as two distinctly separate diseases although the clinical and pathological findings often overlap and one may

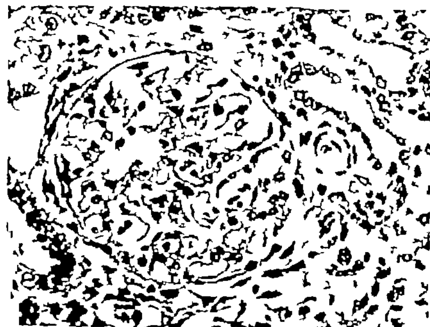


Fig 2 Glomerular corpuscle showing open bulging capillaries, hypercell larity and increased lobulation *The* arterial (arrow) gre ly damaged th characte ric thickening f the w and obliteration of the lu

find the typical TTP microthrombi in patients suffering from both conditions The clinical picture of each attack in our patient shows the typical pattern of HUS while the pathologist describes the findings in the kidney biopsy as TTP

From a review of the literature one may consider the two syndromes as parts of one greater unity (5) the HUS being most often found in children while the TTP is a disease of adult patients still with many exceptions. This view is not yet commonly accepted.

The prognosis of both syndromes is poor (5) but in a few materials a low mortality has been reported (8). The treatment is still symptomatic with blood transfusions and dialysis in the uremic stage while no authors have been able to show a striking effect on survival after treatment with heparin, corticosteroids or immunosuppressive agents (azathioprine) Full recovery has been reported (6, 13) as well as a long chronic course with death in terminal uremia (2, 8) but recurrent relapses with normal kidney function and no proteinuria between the attacks have not been reported earlier

In our patient the immunosuppressive therapy had no effect since new attacks broke out during this treatment.

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